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Water Quality Criteria for 2,4,6-Trinitrotoluene (TNT)

FINAL REPORT

Michael G. Ryon

August 1987

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U.S. ARMY MEDICAL RESEARCH AND
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Fort Detrick, Frederick, MD 21701-5012
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The persistence of TNT in the environment when dissolved in surface water is very limited; biological degradation occurs slowly, while photolytic degradation occurs faster with half-lives for TNT exposed to natural sunlight of 3 to 22 hrs. As a solid or dissolved in groundwater, TNT can remain undecomposed for long periods of time.

Studies of the acute toxicity of TNT to aquatic organisms indicate that LC50 values range from 5.2 to 27.0 mg/L for invertebrates in 48-hr static tests, and from 2.0 to 3.7 mg/L for fish in 96-hr flow-through tests. Chronic studies of early life stages of fish in flow-through conditions indicated egg hatching and fry survival were decreased at levels of 0.84 to 1.35 mg/L in three species exposed for 30 days. In 60-day studies of trout eggs and fry, levels of 0.24 to 1.69 mg/L reduced fry survival. A several-generations study of fathead minnows, conducted under flow-through conditions at levels of 0.04 to 1.21 mg/L TNT, indicated significant effects and affected the

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later generations more severely than the parent generation. Estimates of bioconcentration factors for TNT were not very reliable, but general consensus was that TNT does not highly bioconcentrate.

TNT is absorbed by both humans and test animals through the skin, by ingestion, and by inhalation. Following oral absorption, ^{14}C-TNT is widely distributed throughout the body and is found at highest levels in the GI tract, liver, kidneys, and blood. Studies of the metabolism of TNT have indicated potential pathways and byproducts, particularly products found in urine. The liver is the site for metabolic and detoxification activity. Oral LD_{50}s of TNT in test animals range from 660 mg/kg in mice to 3190 mg/kg in rats. Acute effects after dosing in rats and mice centered on nervous system disturbances with inactivity, tremors, and seizures followed by death. The primary effects of occupational exposure to TNT are jaundice with toxic hepatitis and/or aplastic anemia that can be fatal. Significant effects on the hematological system occurred at mean exposure levels of 0.2 to 7.5 mg/m^3. Evaluations of oral TNT toxicity were reported for 90-day exposures of dogs, mice, and rats. The effects for all three species were similar and included depressed weight gain, mild to moderate anemia, enlarged livers and spleens, some testicular atrophy, and hemosiderosis of the spleen. A similar pattern of effects was found in a 13-wk rat diet study (TNT doses of 1, 5, 25, 125, or 300 mg/kg/day), in a 2-yr rat study (TNT levels of 0.4, 2, 10, and 50 mg/kg/day), and in a 26-wk beagle dog study (given TNT capsules with 0, 0.5, 2.0, 8.0, or 32.0 mg/kg/day). Th Ames *Salmonella typhimurium* assay of pure TNT in strains TA98, TA100, TA1537, and TA1538 demonstrated increased frequencies of mutations, usually in the absence of S9 activating enzymes. Levels of 100 to 1000 mg/plate, 0.5 to 10 g/mL, and 110 to 880 nanomoles/plate produced effects in most strains and frequently in dose-dependent patterns. Carcinogenicity data were limited to a 2-yr study of rats which indicated hyperplasia and carcinoma of the urinary bladder in females at the highest dose (50 mg/kg/day).

Standards for TNT occupational exposures have been recommended by OSHA (TLV of 1.5 mg/m^3), U.S. Army (0.5 mg/m^3), and ACGIH (TLV of 0.5 mg/m^3 and STEL of 0.3 mg/m^3). Drinking water limits of 0.03 to 0.05 mg/L were recommended by the U.S. Army and Navy. Previous calculations of aquatic criteria have been made using the then current USEPA guidelines and for the protection of human health ranged from 6.3 to 44.25 $\mu g/L$ and for the protection of aquatic life ranged from 60 to 900 $\mu g/L$ for the maximum concentrations and 40 $\mu g/L$ for a continuous concentration. Available data for calculating water quality criteria were insufficient to meet all the USEPA guideline requirements. However, enough data were available to calculate a reasonable estimate of the criterion maximum concentration, 557 $\mu g/L$, to protect aquatic life. The other component of the criteria, the criterion continuous concentration, needs further research, but a tentative estimate might be 40 $\mu g/L$. To determine criteria to protect human health, the no-observed-effect level from a chronic study in rats of 0.4 mg/kg/d was used. Application of the proper uncertainity factors, results in an estimate of 135 $\mu g/L$.

ORNL-6304

WATER QUALITY CRITERIA FOR 2,4,6-TRINITROTOLUENE (TNT)

FINAL REPORT

Michael G. Ryon

Chemical Effects Information Task Group
Information Research and Analysis Section
Biology Division

SUPPORTED BY

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EXECUTIVE SUMMARY

2,4,6-Trinitrotoluene (CAS No. 118-96-7) is a flammable solid with the commercial form of yellow monoclinic needles. At room temperature, it is highly stable--detonating only if shocked or heated, has a low vapor pressure, and has very low solubility (about 130 mg/L at 20°C) in water.

The environmental fate of TNT following release into aquatic systems has been well studied. The persistence of TNT in the environment is very limited. Both physical and biological degradation processes affect the residence time of TNT in the environment. Biological degradation by bacterial and fungal species occurs slowly in the environment, with slightly higher rates in the presence of other carbon sources. However, the extent of biological degradation of TNT may not extend to cleavage of the TNT ring. Under suitable conditions, degradation of TNT by photolysis occurs at a much faster rate than by microbial degradation, with half-lives for TNT exposed to natural sunlight of 3 to 22 hrs. Again, the presence of additional organic matter increases the degradation rate. The pH of the water can affect the rate if organic material is at low levels with a lower rate at lower pH. The rate increases with time due to the presence of photolysis products in solution. The pathways of photodegradation of TNT have not been fully elucidated, and several mechanisms involving intermediate compounds have been proposed.

Studies of the acute toxicity of TNT to aquatic organisms indicate that LC_{50} values range from 5.2 to 27.0 mg/L for invertebrates in 48-hr static tests and from 0.8 to 4.2 mg/L for fish in 24- to 96-hr static tests. In 96-hr flow-through tests, the LC_{50} values for fish ranged from 2.0 to 3.7 mg/L. Evaluations of factors that might affect the acute toxicity of TNT indicate that water hardness is not a significant factor, but toxicity decreases with increasing pH, temperature, and photodegradation. By a more sensitive measure, the incipient LC_{50}, values of 0.19 to 13.9 mg/L were found for invertebrates, and values of 1.4 to 1.9 mg/L were found for fish species.

Data on the chronic effects of TNT included an environmental survey of an Army Ammunition Plant (AAP) that produced TNT and several laboratory studies. Wastewater effluents produced effects on macroinvertebrate species numbers, diversity, and density in a reservoir bay downstream from Volunteer AAP. Total munition concentrations (including TNT) of 0.025 mg/L produced no effects on macroinvertebrate communities, but definite effects were noted at 0.150 to 0.600 mg/L with a suggested minimal effect level of 0.050 to 0.100 mg/L. A laboratory microcosm study, using daphnid zooplankton, worms, and algae exposed statically to TNT for 21 days, produced significant reductions in total numbers of daphnids and worms at levels of ≥5.6 mg/L. A 28-day study of effects on daphnids exposed in flow-through conditions to TNT levels of 0.03 to 1.03 mg/L produced no significant effects on survival or reproduction. Some transitory effects (at test days 14 and 21) in lower rates of young produced at 1.03 mg/L might be biologically significant because of the

shorter lifespan of daphnids under natural conditions. Chronic studies of early life stages of three fish species exposed to TNT in flow-through conditions for 30 to 60 days (post-hatch) measured a variety of toxic impacts. Egg hatching and fry survival were decreased at levels of 0.84 to 1.35 mg/L in three fish species exposed for 30 days. In 60-day studies of trout eggs and fry, levels of 0.24 to 1.69 mg/L reduced fry survival, while levels of 0.40 to 0.50 mg/L reduced total length of fry. A several-generations study of fathead minnows conducted under flow-through conditions indicated significant effects based on reproductive and growth indices. Levels of 0.04 to 1.21 mg/L TNT reduced fry growth, lowered hatching rates, lowered survival, and affected the later generations more severely than the parent generation.

Toxic effects of TNT on plants were shown in studies on algae and duckweed species. A level of 1.0 ppm reduced growth and a level of 5.0 ppm produced deaths in duckweed after 11 days of exposure. Algal studies in several species indicated that levels of 2.5 to 9.0 mg/L reduced growth, and levels of 8 to 10 mg/L resulted in death. In a 21-day microcosm study, levels of 5.6 mg/L significantly reduced growth, while levels of 0.6 to 1.0 mg/L indicated transitory or initial effects on growth. The 0.6 mg/L level may be close to the threshold level in this study.

Using data for TNT, Bioconcentration Factors (BCFs) of 9.5 and 338 were calculated for muscle and viscera, respectively, of bluegills. These values were point estimates from the uptake curve and are not highly reliable. Other BCF estimates included 210 to 2030 for four, non-native fish species and a calculated value of 20.5 (based on the octanol water coefficient). The general consensus was that TNT is not a highly bioconcentratable substance.

Trinitrotoluene is absorbed by humans and test animals through the skin, by ingestion, and by inhalation. The amount absorbed varies among different routes and test species; absorption was slower by the dermal route than by the intratracheal and oral routes. Following oral absorption, ^{14}C-TNT is widely distributed throughout the body and is found at highest levels in the GI tract, liver, kidneys, and blood. After 24 hr, the majority of radioactivity from ^{14}C-TNT exposure by any route was found in the urine and feces of all species. Although experiments were not taken beyond 24 hrs, there is an indication that retention of ^{14}C-TNT is not extensive. Because of the low solubility of TNT in water, the radioactivity in urine probably represents metabolic byproducts. No evidence for elimination via exhalation of CO_2 was found.

Studies of the metabolism of TNT have indicated potential pathways and byproducts, particularly products found in urine. Due to the four functional groups on the TNT molecule, several metabolic reactions are possible. These include the primary metabolic pathways of (1) oxidation of the methyl group to alcohol, aldehyde, or carboxyl, and (2) reduction of nitro groups to amino or hydroxylamino compounds (with the possible coupling of these metabolites). The liver is the primary site for this metabolic and detoxification activity.

The oral LD_{50}s of TNT in test animals range from 660 mg/kg in mice to 3190 mg/kg in rats, with most values in the 800 to 1000 mg/kg range. Acute effects after dosing rats and mice centered on nervous system disturbances with inactivity, tremors, and seizures followed by death. Respiratory inhibition, cyanosis, and red-colored urine were also noted.

A large literature base, which documents the effects of occupational exposure to TNT on humans, is available. The primary effects noted are jaundice with toxic hepatitis and aplastic anemia. These effects can occur separately or together and can be fatal. The amount of TNT and the length of exposure required to produce significant effects varied, and the severity of effects appeared to be mostly a matter of individual susceptibility. Other effects detected in occupational exposure studies include gastrointestinal disruptions, hemolytic anemia, contact dermatitis, and cataracts. The effects of TNT exposure on the blood system are variable and may include hemolysis of red cells in the blood stream, destruction of hemoglobin in some erythrocytes, formation of methemoglobin, and formation of sulfahemoglobin. The blood-forming tissues of the marrow are also affected, with a subsequent increase in numbers of reticulocytes in the blood stream and hyperplasia of bone marrow. With further exposure, the bone marrow becomes hypocellular and the number of erythrocytes in the blood are reduced. Progressive aplastic anemia develops with rapid deterioration usually leading to death. Occupational exposure studies indicated that significant effects on the hematological system (primarily decreased hemoglobin) occurred at mean exposure levels of 0.2 to 7.5 mg/m^3.

Evaluations of oral TNT toxicity were reported, based on 90-day exposures of dogs, mice, and rats. The dogs were given TNT dose levels of 0, 0.2, 2.0, or 20 mg/kg/day in gelatin capsules. Levels of 0, 0.002, 0.01, 0.05, or 0.25 percent TNT by weight in the diet were fed to rats. Levels of 0, 0.001, 0.005, 0.025, or 0.125 percent TNT were fed to mice. In general, the effects for all three species were similar, with a little more severity in dogs and rats. Principal effects included depressed weight gain, mild to moderate anemia, enlarged livers and spleens, some testicular atrophy, and hemosiderosis of the spleen. These studies parallel many of the human case history reports, indicting anemia (usually hemolytic, but also aplastic) and liver damage as the primary responses. A similar pattern of effects was found in a 13-wk rat diet study (TNT doses of 1, 5, 25, 125, or 300 mg/kg/day), in a 2-yr rat study (TNT levels of 0.4, 2, 10, and 50 mg/kg/day), and in a 26-wk beagle dog study (given TNT capsules with 0, 0.5, 2.0, 8.0, or 32.0 mg/kg/day). The pattern of anemic effects indicated a more hemolytic basis than an aplastic anemia.

A variety of short-term test systems has been used to evaluate the genotoxicity of TNT and TNT-complexes with most systems indicating positive effects. The Ames *Salmonella typhimurium* assay was used to test TNT and the urine of workers in TNT production facilities. Tests of pure TNT in *S. typhimurium* strains TA98, TA100, TA1537, and TA1538 demonstrated increased frequencies of mutations, usually in the absence of S9 activating enzymes. Levels of 100 to 1000 mg/plate, 0.5 to 10 g/mL, and 110 to 880 nanomoles/plate produced effects in most strains

and frequently in dose-dependent patterns. A study of Swedish TNT workers evaluated genotoxicity with a bacterial fluctuation assay which indicates increased frameshift mutations when tested without S9 activation. Urine samples from 14 workers produced significant increases ($p < 0.01$), and these increases could be correlated with observed TNT levels in the ambient air of the work stations. Other evaluations of genotoxicity of TNT included a cytogenic analysis of bone marrow, unscheduled DNA synthesis (UDS) assays in rat liver cells and human diploid fibroblasts, a P388 mouse lymphoma gene mutation assay, and a mouse bone marrow micronucleus assay. Only in the human cell UDS assay (at 1000 g/mL) and the mouse lymphoma assay (at doses from 0 to 1000 g/mL) were significant genotoxic effects reported.

No data were found on the teratogenic or developmental toxicity of TNT in mammals. The only indications of reproductive effects were scattered effects on testes of rodents in chronic tests. Carcinogenicity data were limited to a 2-yr study of rats, which indicated hyperplasia and carcinoma of the urinary bladder in females at the highest dose (50 mg/kg/day).

Standards for TNT occupational exposures have been recommended by OSHA (TLV of 1.5 mg/m^3), U.S. Army (0.5 mg/m^3), and ACGIH (TLV of 0.5 mg/m^3 and STEL of 0.3 mg/m^3). Drinking water limits of 0.03 to 0.05 mg/L were recommended by the U.S. Army and Navy. Previous calculations of aquatic criteria have been made using the then current USEPA guidelines and for the protection of human health ranged from 6.3 to 44 $\mu g/L$ and for the protection of aquatic life ranged from 60 to 900 $\mu g/L$ for the maximum concentrations and 40 $\mu g/L$ for a continuous concentration.

Available data for calculating water quality criteria were insufficient to meet all the USEPA guideline requirements. However, enough data were available to permit calculation of a reasonable estimate of the criterion maximum concentration, 557 $\mu g/L$, to protect aquatic life. The other component of the criteria, the criterion continuous concentration, needs further research, but a tentative estimate might be 40 $\mu g/L$. To determine criteria to protect human health, the no-observed-effect level from a chronic study in rats of 0.4 mg/kg/day was used. Application of the proper uncertainity factors results in an estimate of 135 $\mu g/L$. All the criteria generated in this report are generally of the same magnitude as prior calculations of aquatic criteria.

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1. INTRODUCTION

2,4,6-Trinitrotoluene, also known as α-trinitrotoluene or TNT, is a munitions compound currently and previously manufactured or handled at several U.S. Army facilities, including Radford Army Ammunition Plant (AAP), Volunteer AAP, Alabama AAP, Holston AAP, Iowa AAP, Joliet AAP, Lone Star AAP, McAlester AAP, Milan AAP, and Newport AAP (Pal and Ryon 1985). Although the majority of current TNT manufacture occurs at Radford AAP, Load Assemble and Pack (LAP) activities occur at several of the other AAPs, and other sites contain residual contamination in soils, groundwaters, and treatment lagoons that may be potentially hazardous. The objective of this report is to review the available data on the aquatic and human health effects of TNT and, using the latest U.S. Environmental Protection Agency (USEPA) guidelines, generate water quality criteria values. Appendix A is a summary of the USEPA guidelines for generating water quality criteria for the protection of aquatic organisms and their uses (Stephan et al. 1985). Appendix B is a summary of the USEPA guidelines for generating water quality criteria for the protection of human health (USEPA 1980).

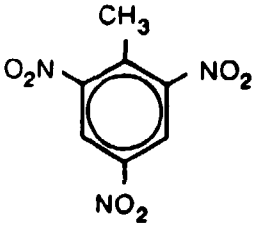
1.1 CHEMICAL IDENTITY AND PROPERTIES

2,4,6-Trinitrotoluene (CAS No. 118-96-7) is a flammable solid with the commercial form of yellow monoclinic needles (ACGIH 1983). At room temperature it is highly stable (detonating only if shocked or heated), has a low vapor pressure, and is virtually insoluble in water. Synonyms and a summary of chemical and physical properties are given below with data from Lindner (1980), unless indicated otherwise.

Molecular formula: $C_7H_5N_3O_6$

Molecular weight: 227.13

Structural formula:



CAS registry number: 118-96-7; RETCS (1983-1984) No. XV0175000

Chemical name: 2,4,6-Trinitrotoluene

Synonyms: TNT, 2-methyl-1,3,5-trinitrobenzene, α-trinitrotoluol, sym-trinitrotoluene, 1-methyl-2,4,6-trinitrobenzene, Tolit, Trilit, Trotyl, tritol, sym-trinitrotoluol (Windholz 1976); Entsufon, s-trinitrotoluene [MEDLARS (RTECS) 1985]

Elemental analysis: C, 37.01 percent; H, 2.22 percent; N, 18.50 percent; O, 42.27 percent (Windholz 1976)

Melting point: 80.1°C (Windholz 1976)

Boiling Point: 190°C at 2 mm Hg, 245-250°C at 50 mm Hg (Urbanski 1983b)

Density: D_4^{20} 1.654 (solid) (Windholz 1976); 1.467 at 82°C (Urbanski 1983b)

$D(g/cm^3) = 1.5446 - 1.016 \times 10^{-3} t$, where t = °C; applicable in the temperature range 83°-120°

Dipole moment: 1.37D (Windholz 1976)

Hygroscopicity: Practically nonhygroscopic, 0.03 percent at 30°C and 90 percent humidity (Zakhari et al. 1978)

Vapor pressure: 0.046 mm Hg at 82°C [MEDLARS (CTDB) 1985]

Stability: TNT is very stable and may be stored indefinitely at temperate conditions without deterioration.

Decomposition: The decomposition mechanism of TNT at elevated temperatures (200°C) is very complex, producing at least 25 different compounds as well as large amounts of undefined polymeric material.

Viscosity: 8 cP at 99°C; 9.5 cP at 100°C (Zakhari et al. 1978)

Detonation products (experimentally determined in a calorimetric bomb, mole per mole TNT): 3.65 C (sol), 1.98 CO, 1.60 H_2O, 1.32 N_2, 0.46 H_2, 0.16 NH_3, and 0.10 CH_4

Heat of formation, kJ/g: 0.293

Heat of combustion, kJ/g: 15.02

Heat of detonation, kJ/g: 4.23

Specific heat, J/(g × K): 1.38

Heat of fusion, J/g: 98.3

Heat of vaporization, J/g: 339

Heat of sublimation, J/g: 447

Thermal conductivity, W/(m·K): 0.54

Coefficient of linear expansion: $6.7 \times 10^{-3}/°C$

Solubility (g/100 g)in water (Zakhari et al. 1978):

0.010 at 0°C	0.068 at 60°C
0.013 at 20°C	0.148 at 100°C
0.029 at 40°C	

Solubility (g/100 g) in other solvents at 20°C (Windholz 1976):

Pyridine - 137	1,2-Dichloroethane - 18.7
Acetone - 109	Diethyl ether - 3.29
Toluene - 55.0	Trichloromethylene - 3.04
Benzene - 67.0	Ethanol (95 percent) - 1.23
Methyl acetate - 72.1	Carbon tetrachloride - 0.65
Chlorobenzene - 33.9	Carbon disulfide - 0.48
Chloroform - 19.0	Ethylene dichloride - 18.7

Log octanol-water partition coefficient: 1.84
(method of Lyman et al. 1981, as used by Rosenblatt 1981).

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

Traditionally, TNT has been manufactured by nitrating toluene in several stages with a mixture of H_2SO_4 and HNO_3 as the nitrating agent. Improvements in TNT manufacturing and purification processes have taken place, but the basic principle has not changed (Urbanski 1983a).

A major improvement in the commercial manufacture of TNT was the replacement of the batch process by the continuous method of manufacture (Urbanski 1983b). The British method of continuous manufacture of TNT was developed in 1917, while a continuous method developed by J. Meisner was perfected in Germany during World War II. A 1953 study group concluded, after a survey of the seven known European continuous processes, that the Bofors-Norell process was the best (Gilbert 1980, citing data of Wendes and Little 1954). This process includes both continuous nitration of toluene or mononitrotoluene to trinitrotoluene and continuous crystallization of the product (TNT) from dilute nitric acid (Urbanski 1983b). A second study group in 1967 selected the Swedish Nobel-Chematur Process as modified by Canadian Industry Ltd. (using sodium sulfite instead of nitric acid in the purification step), as the best available continuous process (Gilbert 1980, citing data of Slemrod 1970). It was a further modification of this process that resulted in the first continuous operation of TNT manufacture in the United States in 1968 at Radford AAP.

A wide variety of analytical techniques have been used to identify TNT in process streams, vapor emissions, and wastewater effluents. These techniques include wet chemical methods (usually combined with more sophisticated instrumental techniques), electrochemical methods, chromatography, and spectrophotometry; the topic is reviewed by Castorina (1980), and because the area is so large, only a few examples from his review will be mentioned. One electrochemical technique, single sweep polarography, has determined TNT concentrations in water as

low as 50 ppb and is applicable to both field and laboratory conditions. Gas chromatography (GC) used in conjunction with thin-layer chromatography can give quantitative analyses of TNT and biodegradation products of TNT in the micro- to picogram range. Wastewaters from Army TNT manufacturing plants have been analyzed for TNT and degradation products by GC, thin-layer chromatography, high performance liquid chromatography (with an ultraviolet [UV] detector at 220 nm), and column chromatography. A spectrophotometric method has been used on-line to monitor carbon columns in wastewater treatment for TNT at concentrations as low as 0.1 ppm. Also, a fluorescent-dyed quaternary ammonium ion exchange resin has been used to detect TNT at 70 ppb levels in wastewaters after irradiation with UV. A colorimetric dual channel system was used to detect TNT in wastewater effluents (at levels of 1 ppm) and when compared to GC methods gave a better analysis of other TNT isomers and degradation products, was easier to use, and was more suitable for routine surveillance. Spectrometric methods that have been used for TNT include infrared, nuclear magnetic resonance, X-ray, mass, negative-ion mass, plasma chromatography mass (detected TNT in air at 10 pp trillion), and chemical ionization mass. More applications of these and other techniques are also reviewed by Castorina (1980) but are beyond the scope of this document.

2. ENVIRONMENTAL EFFECTS AND FATE

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

Information on the effects of TNT on water quality consists primarily of data generated from various AAPs. Studies were made at Radford AAP (Huff et al. 1975a), Volunteer AAP (Huff et al. 1975b, Sullivan et al. 1977), Joliet AAP (Ghassemi et al. 1976; Stilwell et al. 1976; Jerger et al. 1976), Iowa AAP (Weitzel et al. 1975; Jerger et al. 1976; Sanocki et al. 1976), and Alabama AAP (Keirn et al. 1981) measuring various physical and chemical parameters of the affected aquatic systems relative to distance from the AAP or to actual levels of TNT in the water or sediments. These data are quite variable because many factors affect water quality, including the munition production levels and conditions, weather conditions, and time of year. Also, other related compounds (e.g., dinitrotoluenes) were present in most of the wastewater effluents, complicating the analysis. A more detailed review of these studies can be found in Ryon et al. (1984).

In general, the effects of TNT wastewater on water quality are concentrated on several parameters. TNT wastewater increased the chemical oxygen demand (COD), the load of solids (both dissolved and suspended), the levels of nitrogen species (nitrates, nitrites, and total Kjeldahl nitrogen), and the levels of sulfates in the water of receiving streams at a majority of AAP locations. Affected to a lesser degree were total organic carbon, pH, and phosphate levels. The effects on associated sediments paralleled those found for the water column, with the greatest impact on COD, levels of nitrogen species, and phosphate concentrations. The expected significance of these effects would be to increase nutrients for algal and plant growth, reduce light penetration and substrate habitat availability due to input of solids, reduce oxygen availability for animal utilization and organic decomposition, and, in general, increase the frequency of changes in water parameters (e.g., in pH) that are stressful to the biotic communities. However, these studies do not provide a conclusive link between TNT and these effects, because of the presence of many other compounds in the wastewaters discharged from the AAPs.

2.2 ENVIRONMENTAL FATE

Persistence of munition compounds in the environment is dependent on several factors including the degree to which they bioaccumulate and the processes that degrade them. This section will first discuss the overall question of persistence and then examine in more detail some of the individual factors involved.

2.2.1 Persistence of TNT

Solid chunks of TNT, buried or on the surface of soil, can persist for many years (Rosenblatt 1986 pers. comm.).

In the aquatic environment, mechanisms affecting persistence include the physical degradation processes of photolysis and hydrolysis as well as biological degradation by bacteria. A laboratory study of the impact of photolysis and microbial degradation on TNT was reported by Spanggord et al. (1980). Both processes were important to the degradation of TNT, but in natural waters biotransformation was considerably slower (under certain conditions as much as 1,000 times slower) than photolysis. The photolysis half-lives of TNT vary with season and latitude, as shown in Table 1. Studies of microbial degradation indicate that biodegradation half-lives are longer (19 to 25 days) than the half-lives of photolysis and occur after a lag period (13 to 20 days). The rate of transformation is increased by the presence of additional organic or nutritional material. Simulation analyses based on data from AAPs also indicate that in natural waters the photolysis half-lives for TNT are short (3 to 22 hr), while the microbial degradation half-lives are comparatively long (21 to 65 days, including acclimation period). A study of natural Potomac River water spiked with 20 mg/L TNT and microbial sediments under exposure conditions of sunlight, shade, or darkness found complete disappearance of TNT in 6 to 8 days in sunlight, while 730 days were required for 90 percent disappearance in shade (Burlinson 1980).

TABLE 1. SUNLIGHT PHOTOLYSIS OF TNT[a] IN PURE WATER[b]

Latitude (°N)	Summer Half-life (hr)	Winter Half-life (hr)
20	14	22
40	14	45
50	14	84

a. TNT = 2,4,6-trinitrotoluene.
b. Adapted from Spanggord et al. 1980.

Data on the persistence of TNT in the aquatic environment were provided by Jerger et al. (1976). They used sediments from Iowa and Joliet AAPs as microbial sources for degradation studies. In laboratory microcosm studies, Jerger et al. (1976) found that 90 percent of a 10 ppm TNT solution was transformed following three days of incubation.

Sikka et al. (1980) studied the sorption and desorption of TNT by sediments. They used 0.5 g of nonsterilized sediments from a farm pond and the Holston River (above the Holston AAP) and 50 mL of ^{14}C-TNT solution in 125 mL Erlenmeyer flasks. The flasks were shaken at enough speed to maintain the sediment in suspension, were kept in the dark, and were kept at a constant temperature (30°C). One-mL samples were taken at various intervals over a 24-hr period and tested to determine TNT concentration; changes in concentrations were assumed to be due to sorption by the sediments. Desorption studies were also done using the

sediments from earlier sorption studies. The sediments were suspended in fresh phosphate buffer (pH 7) 72 hrs after completion of the sorption studies, and samples were taken to determine any change in soluble TNT concentrations. Sikka et al. (1980) found that sorption increased with time at initial solution concentrations of 0.2 to 30.2 mg/L and was linear with increasing TNT concentrations. Partition coefficients ranged from 5.5 to 22.2 for the four sediments tested. Partition coefficients varied with pH (3.8, 7.0, and 8.9) and temperature (10°, 20°, and 30°C), being lowest at a pH of 3.8 or a temperature of 10°C. The desorption studies demonstrated that TNT or TNT breakdown products were released slowly from the sediments, with the equilibrium reached only after 92 hrs. In general, Sikka et al. concluded that sediment sorption of TNT is very low, but once bound by the sediments release is very slow. Evidently TNT can also persist for long periods in groundwater (Rosenblatt 1986 pers. comm.)

2.2.2 Biological Degradation of TNT

As previously indicated, TNT and compounds associated with its production can be degraded and transformed by bacterial and fungal species in aquatic environments. The bacterial species that have been identified as effective degraders of TNT are shown in Table 2 along with the resulting degradation products. Mechanisms by which the degradation occurs have been proposed by several authors.

It is generally agreed that degradation of TNT occurs fastest in the presence of added nutrients. Adding varying levels of glucose speeded TNT transformation (Osmon and Klausmeier 1972; Won et al. 1974; Weitzel et al. 1975; Jerger et al. 1976; Amerkhanova and Naumova 1978; Hoffsommer et al. 1978), and adding yeast also produced increased transformation of TNT with reported rates of 100 mg/L in 6 days (Osmon and Klausmeier 1972) and 100 μg/mL in 24 hrs (Won et al. 1974). The transformation rate was shown to increase as the yeast concentration increased (Spanggord et al. 1980). Transformation of TNT occurred more slowly when it was the sole source of carbon (Weitzel et al. 1975; Traxler 1975; Spanggord et al. 1980); only by using population densities in excess of 7.5 mg cells/mL (far above normal environmental levels) could rapid transformation rates (90 percent in 24 hrs) be achieved (Traxler 1975). The increase in metabolic rate with the addition of nutrients indicates that co-metabolism may be occurring in the degradation of TNT.

The extent of reported structural degradation of TNT was found to vary. Breakdown of the ring structure was not found by some researchers (Hoffsommer et al. 1978; Isbister et al. 1980; Spanggord et al 1980), while ring cleavage was indicated by others (Traxler 1975; Hale et al. 1979). Both of these groups used ^{14}C-labels to identify degradation products. Unfortunately, in the studies indicating ring cleavage, the effects of photolysis were unclear. Hale et al. mentioned this uncertainty, but no reference to it was made in the Traxler studies. A personal communication from Dr. David Kaplan (1985) indicates that the current consensus of researchers in this area supports the position that the ring is not cleaved; partly because the work indicating cleavage was

TABLE 2. BACTERIAL SPECIES AND METABOLITES IN BIODEGRADATION STUDIES OF TNT AND ASSOCIATED COMPOUNDS

Munition[a]	Species/Source	Metabolites Identified	Reference
Synthetic	Azobacter agilis	NG[b]	Bringmann and Kuehn 1971
TNT	Bacillus subtilis	hydroxylaminotoluene; aminotoluene	Klausmeier et al. 1976
TNT	Bacillus sp.	NG	Yang et al. 1979
TNT	Citrobacter sp.	NG	Yang et al. 1979
TNT	Clostridium plasterianum	2,6-dinitro-4-hydroxylaminotoluene	McCormick et al. 1976
TNT	Enterobacter sp.	NG	Yang et al. 1979
TNT	Escherichia coli	4-amino-2,6-dinitrotoluene; 4,6-dinitro-2-aminotoluene	Hudock 1972[c]
TNT	Escherichia coli	NG	Amerkhanova and Naumova 1975
TNT	Escherichia coli	2,2',6,6'-tetranitro-4,4'-azoxytoluene[d]; 2,4-diamino-6-nitrotoluene[e]	McCormick et al. 1976
TNT	Escherichia coli	NG	Amerkhanova and Naumova 1978
TNT	Escherichia sp.	NG	Yang et al. 1979
TNT	Klebsiella sp.	NG	Yang et al. 1979
TNT	Pseudomonas aeruginosa	NG	Osmon and Klausmeier 1972
TNT	Pseudonomas aeruginosa	hydroxylaminotoluene; aminotoluene	Klausmeier et al. 1976
TNT	Pseudonomas denitrificans	NG	Amerkhanova and Naumova 1978
TNT	Pseudonomas denitrificans	4-amino-2,6-dinitrotoluene; 4,6-dinitro-2-aminotoluene	Hudock 1972[c]
TNT	Pseudonomas fluorescens	4-amino-2,6-dinitrotoluene; 4,6-dinitro-2-aminotoluene	Hudock 1972[c]
TNT	Pseudomonas FR2	2,2',6,6'-tetranitro-4,4'-azoxytoluene; 2,4-diamino-6-nitrotoluene; 4-amino-2,6-dinitrotoluene	McCormick et al. 1976
TNT	Pseudonomas putida	4-amino-2,6-dinitrotoluene; 4,6-dinitro-2-aminotoluene	Hudock 1972[c]
TNT	Pseudomonas striata	NG	Amerkhanova and Naumova 1975
TNT	Pseudomonas sp. I	NG	Amerkhanova and Naumova 1975

TABLE 2. CONTINUED

Munition[a]	Species/Source	Metabolites Identified	Reference
TNT	Pseudonomas putida	4-amino-2,6-dinitrotoluene; 4,6-dinitro-2-aminotoluene	Hudock 1972[c]
TNT	Pseudomonas striata	NG	Amerkhanova and Naumova 1975
TNT	Pseudomonas sp. I	NG	Amerkhanova and Naumova 1975
TNT	Pseudomonas spp.	2,2',4,4'-tetranitro-4-azoxytoluene; 2,2',4,4'-tetranitro-6-azoxytoluene; 4,6-dinitro-2-aminotoluene; 2,6-dinitro-4-hydroxylaminotoluene; nitrodiaminotoluene	Won et al. 1974
TNT	Veillonella alkalescens	2,4-diamino-6-nitrotoluene	McCormick et al. 1976
TNT	Sediment Population[f,g]	hydroxyamino-dinitrotoluene	Weitzel et al. 1975
TNT	Sediment Population[f,h]	hydroxyamino-dinitrotoluene	Jerger et al. 1976
TNT	Sediment population[i]	4,6-dinitro-2-aminotoluene; 4-amino-2,6-dinitrotoluene; 2,4-diamino-6-nitrotoluene; 2,6-diamino-4-nitrotoluene; 2,2',6,6'-tetranitro-4,4'-azoxytoluene; 4,4',6,6'-tetranitro-2,2'-azoxytoluene	Spanggord et al. 1980
TNT	Sediment Population[j]	4-amino-2,6-dinitrotoluene; 2-amino-4,6-dinitrotoluene; 3,5-dinitroaniline	Burlinson 1980
TNT	Sludge population[k]	4-amino-2,6-dinitrotoluene; 4,6-dinitro-2-aminotoluene; 2,4-diamino-6-nitrotoluene; 2,6-diamino-4-nitrotoluene	Hoffsommer et al. 1978
TNT	Soil populations	4-amino-dinitrotoluene	Osmon and Andrews 1978

a. TNT = 2,4,6-trinitrotoluene; Synthetic wastewater contained 2,4,6-trinitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 2-nitrotoluene, 4-nitrotoluene, 1,3,5-trinitrobenzene, m-dinitrobenzene, and nitrobenzene.
b. NG = not given.
c. As cited in Hoffsommer et al. 1978.
d. Aerobic and anaerobic conditions.
e. Aerobic conditions only.
f. Intermediate metabolites.
g. Aerobic bacterial population from sediments at Iowa AAP.
h. Aerobic bacterial population from sediments at Iowa and Joliet AAPs.
i. Aerobic sediment population from Waconda Bay downstream from Volunteer AAP.
j. Aerobic sediment population from Potomac River sediments.
k. Bacterial population from an aerobic activated sludge source.

not substantiated by later work and some problems were identified with the original studies. Utilization of the nitro groups in TNT by bacteria was more universally accepted (Traxler 1975; Amerkhanova and Naumova 1978; Spanggord et al. 1980).

The pathways for degradation of TNT have been discussed by several authors. Won et al. (1974) found that TNT is reduced within 24 hours to form 2,2',6,6'-tetranitro-4-azoxytoluene, 2,2'4,4'-tetranitro-6-azoxytoluene, 2,6-dinitro-4-hydroxylaminotoluene and its isomer 2,4-dinitro-6-hydroxylaminotoluene. After depletion of TNT, the azoxy compounds degrade. The other two compounds are then reduced to the respective 4-amino and 2-amino compounds. These are further reduced to nitrodiaminotoluene. McCormick et al. (1976) elaborated on these pathways, suggesting: (1) the reduction of a nitro group to an amino group (Figure 1) followed by oxidative deamination to a phenol, releasing ammonia; and (2) release of a nitro group as nitrite with the formation of a phenol. Hoffsommer et al. (1978) reported the metabolic reduction possibilities of TNT in an open treatment ditch. They found evidence for the stepwise nitro reduction process outlined by McCormick et al., including the formation of the tetranitroazoxy compounds. Hoffsommer et al. also found formation of insoluble ^{14}C-labelled products that indicate the presence of 2,4,6-triaminotoluene. Spanggord et al. (1980) also supported these proposed degradation pathways and indicated that there is no reduction preference between either the 2- or 4- position, so there should be equal amounts of the amino-dinitrotoluene isomers and the nitroazoxytoluenes.

Biological reduction of TNT was reported by Parrish (1977) for 190 species of fungi. He found that with glucose added, 183 species could reduce TNT. The transformation products of TNT that were identified included 4-amino-2,6-dinitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene, and 2,2',6,6'-tetranitro-4,4'-azoxytoluene. Using ^{14}C-labelled TNT, he could not find evidence of ring cleavage.

2.2.3 Physical Degradation of TNT

The primary physical mechanism that degrades TNT munitions in exposed aqueous solution is photolysis. Exposure to sunlight or any source of UV light produces fairly rapid breakdown of the TNT and its associated compounds, including many impurities and resulting degradation products. Generally, UV light with a wavelength below 420 nm degrades TNT directly. Wavelengths between 400 and 500 nm degrade TNT indirectly through solar-absorption by natural products and energy transfer to the TNT (Spanggord et al. 1980).

Andrews and Osmon (1976) found that 1,000 ppm of 1,3,5-TNT in aqueous solution was reduced to 170 ppb after 24 hrs exposure to 240-260 nm UV light. This produced a light pink residual in the water, indicating continued presence of aromatic compounds, but ring cleavage to CO_2 and volatile organics was also shown. A pink water solution containing 221 ppm of TNT was also degraded after 49 hrs under optimum UV-exposure conditions. The half-lives of TNT exposed to sunlight in natural water

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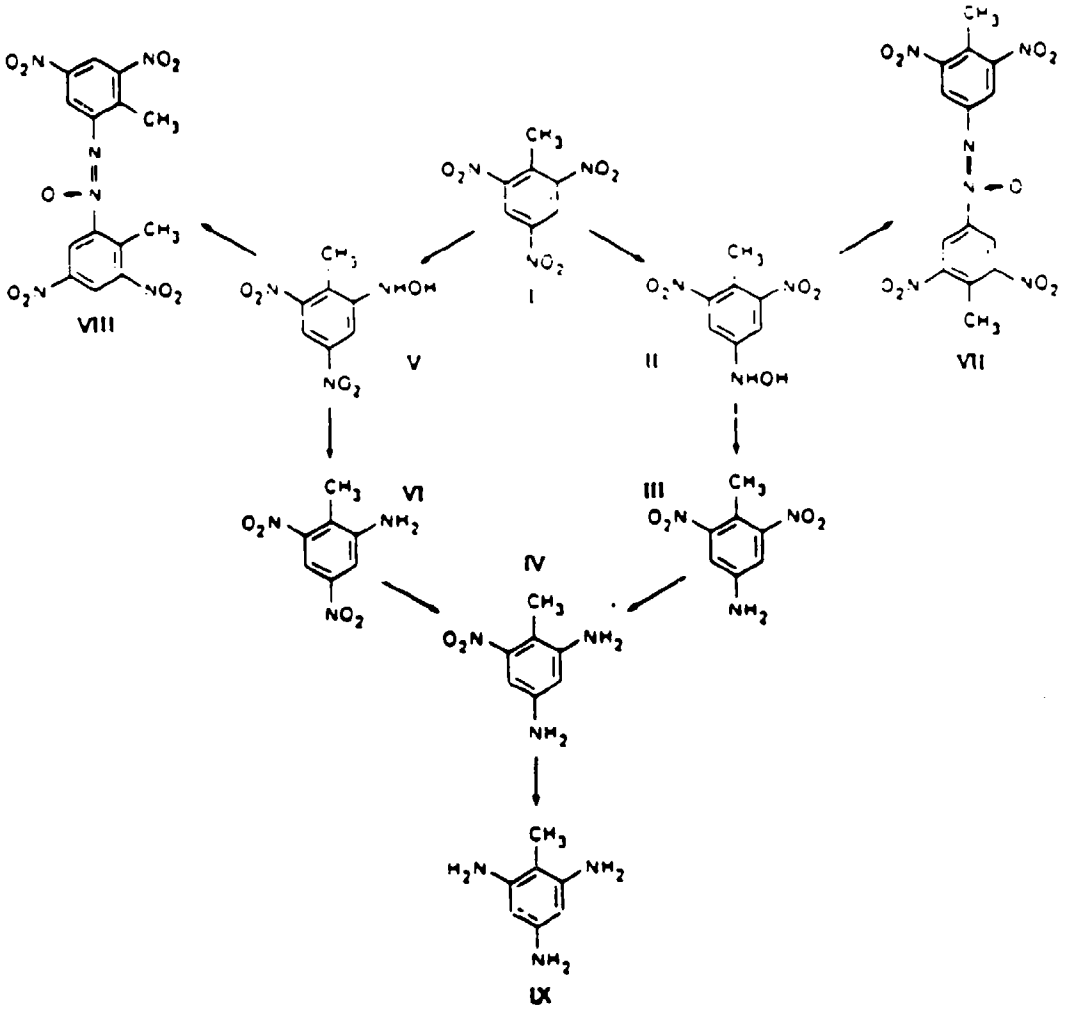

Figure 1. Proposed pathways for transformation of TNT by reduction of the proposed nitro groups. Compounds illustrated are: I = 2,4,6-trinitrotoluene; II = 4-hydroxylamino-2,6-dinitrotoluene; III = 4-amino-2,6-dinitrotoluene; IV = 2,4-diamino-6-nitrotoluene; V = 2-hydroxylamino-4,6-dinitrotoluene; VI = 2-amino-4,6-dinitrotoluene; VII = 2,2,6,6'-tetranitro-4,4'-azoxytoluene; VIII = 4,4',6,6'-tetranitro-2,2'-azoxytoluene; and IX = 2,4,6-triaminotoluene. From McCormick et al. 1976.

ranged from 3 to 22 hours (Spanggord et al. 1980), and photolysis rate constants [$K_p^1(S^{-1}) \times 10^4$] ranged from 4.88 to 16.7 for natural water (Spanggord et al. 1981).

The conditions under which aqueous solutions of TNT are exposed to sunlight can affect the photolysis process. Solutions that contain organic matter result in faster photolysis rates than solutions in distilled water (Spanggord et al. 1980). The pH of the solution can be a factor in the rate of photolysis if the water contains little organic matter (Burlinson et al. 1973, as cited in Isbister et al. 1980; Spanggord et al. 1980) and generally, lower rates are produced at lower pHs. The rate also increases with time, a result of co-metabolism due to the increase in photolysis products in solution (Spanggord et al. 1980).

The pathway by which TNT is reduced has not been fully elucidated (Mabey et al. 1983; Spanggord et al. 1980). Intermediate compounds have been identified and attributed to photolysis (Table 3), but the details of their formation are only generally known. Several authors have suggested that the principal information supports a degradation process via a triplet-sensitized mechanism (Rosenblatt et al. 1971; Spanggord et al. 1980; Mabey et al. 1983). This mechanism postulates formation of a triplet excited state of the TNT compound similar to the one in Figure 2 which permits a weak nucleophile to attack and remove an NO_2^- or X^- group (Rosenblatt et al. 1971). Another proposed mechanism is based on the formation of a complex between humic substances in water and the TNT compound (Spanggord et al. 1981). The presence of such a complex would alter the UV absorbencies responsible for photodegradation of TNT. Spanggord et al. (1981) tested the possibility of a TNT-humic acid complex by using a polarographic technique to determine if the "free" TNT in pure water had a different reduction potential than TNT in water from Searsville pond (high in organic substances). They found differences in the reduction polargrams between the two TNT/water combinations, indicating the possible formation of a TNT-humic acid complex. Theoretically, such a complex might absorb sunlight more readily than TNT alone, leading to faster photodegradation.

2.3 SUMMARY

A clear cause and effect relationship for abiotic environmental effects of TNT has not been demonstrated. Many environmental surveys and reports of the effects of TNT wastewater discharged from production and handling facilities of the U.S. Army have demonstrated that the chemical complex in the wastewater does produce effects on COD, solids load, nitrogen species levels, and sulfate levels. However, many chemicals have the potential to cause these effects, and the data do not specifically indicate TNT as the causative agent.

The environmental fate of TNT following discharge into aquatic systems has been well studied. The persistence of TNT in the environment is very limited, as both physical and biological degradation processes affect the residence time of TNT in the environment. Biological degradation by bacterial and fungal species occurs slowly in the environment,

TABLE 3. KNOWN PHOTOLYSIS PRODUCTS OF 2,4,6-TRINITROTOLUENE[a]

1,3,5-Trinitrobenzene
1,3-Dinitrobenzene
2,4-Dinitrotoluene
2,4,6-Trinitrobenzaldehyde
2,4,6-Trinitrobenzyl alcohol
2,4,6-Trinitrobenzonitrile
2,4,6-Trinitrobenzaldoxime
4,6-Dinitro-1,2-benzisoxazole
4,6-Dinitroanthranil
3,5-Dinitrophenol
2,4,6-Trinitrobenzoic acid
2-Amino-4,6-dinitrobenzoic acid
2,2'-Dicarboxy-3,3',5,5'-tetranitroazoxybenzene
2,2'-Dicarboxy-3,3',5,5'-tetranitroazobenzene
2-Carboxy-3,3',5,5'-tetranitroazoxybenzene
N-(2-Carboxy-3,5-dinitrophenyl)-2,4,6-trinitrobenzamide

a. Adapted from Jerger et al. 1976.

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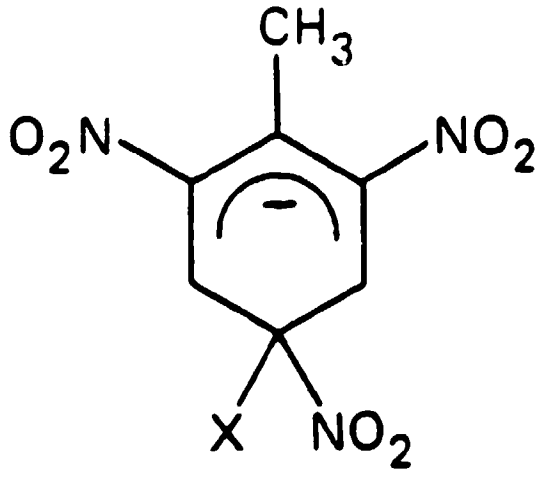

Figure 2. Proposed excited state intermediate of 2,4,6-Trinitrotoluene found in photolytic reactions. From Rosenblatt et al. 1971.

with slightly higher rates in the presence of other carbon sources. However, the extent of biological degradation of TNT may not extend to cleavage of the TNT ring. Degradation of TNT by photolysis can occur at a much faster rate than by microbial degradation in most natural waters, with half-lives for TNT exposed to natural sunlight of 3 to 22 hrs. Again, the presence of additional organic matter increases the degradation rate. The pH of the water can affect the rate if organic material is at low levels. The rate increases with time due to the presence of photolysis products in solution. The pathways of photodegradation of TNT have not been fully elucidated, and several mechanisms involving intermediate compounds have been proposed.

3. AQUATIC TOXICOLOGY

3.1 ACUTE TOXICITY TO ANIMALS

Studies of the acute toxicity of TNT to aquatic animals were performed on several different families of invertebrates and fish. Generally, the results are presented as standard LC50 values (Table 4); but additionally, effects were noted for the influence of water conditions on the toxicity of TNT.

Pederson (1970) exposed groups of 10 *Lepomis macrochirus* to TNT solutions for 96 hr in 12-quart polyethylene buckets containing 10 L of water. Preliminary studies indicated a decrease in TNT concentration due to photodegradation after 24 hr, so the TNT solutions were renewed on a 24-hr basis. Exposure conditions were varied in order to evaluate the effects of temperature and water hardness on the LC50 values of TNT. Tested conditions included two temperatures (10° 25°C) and two levels of water hardness (60 and 180 ppm as CaCO3). The resulting LC50 values indicated that water hardness was not a factor, but temperature was with the toxicity at 10°C being slightly lower than at 25°C (Table 4).

A study by Liu et al. (1976) used *Pimephales promelas* and *Daphnia magna* to determine the LC50 values of TNT and evaluate the effects of pH and ultraviolet photodegradation on those values. The static tests were performed according to USEPA (1975) guidelines with the minnows placed in 19-L jars containing 15 L of test solution, and the invertebrates placed in 250-mL beakers containing 200 mL of solution. The LC50 values determined for the standard conditions (pH 7 and 0 percent degradation) are given in Table 4, with 48-hr values for the invertebrates and 96-hr values for the minnows. The evaluation of pH and photodegradation effects were performed only with *P. promelas*. The pH levels tested included 5, 7, and 9, and the extent of photodegradation included 0, 50, and 100 percent. The LC50 values determined for the varied conditions are given in Table 5. These tests indicated that after 96 hr, the toxicity of TNT to *P. promelas* decreased with increasing pH and that photodegradation of the TNT solutions reduced the toxicity.

Another evaluation of the acute toxicity of TNT to *P. promelas* was performed by Smock et al. (1976); however, this bioassay was designed as a flow-through test with complete replacement of the TNT test solution in a 15-L tank every 8 hr. The concentrations were initially determined and monitored throughout the 96-hr test by spectrophotometric analysis. Death was used as an indication of toxicity for the LC50 determinations, and behavioral responses were used to determine the median effective concentration (EC50). The LC50 value was 2.58 mg/L and the EC50 value was 0.46 mg/L (Table 4). The lower EC50 value shows that TNT produced initial behavioral effects at a much lower concentration than the lethal concentration, and observations indicated that these effects occurred within the first few minutes of exposure. The effects included a shock reaction (gasping at the surface and lethargy), loss of motor control (jerky swimming motions with exaggerated gill movements), and a yawing,

TABLE 4. ACUTE TESTS FOR MORTALITY OR IMMOBILIZATION[a] OF AQUATIC SPECIES FOLLOWING EXPOSURE TO 2,4,6-TRINITROTOLUENE

Test Species	Test Method[b]	Test Duration (hr)	LC_{50} mg/L (CI)[c]	Genus Mean Acute Value (mg/L)[d]	Reference
Arthropoda					
Crustacea					
Daphnidae					
Daphnia magna[e]	S	48	6.6 (4.5-9.7)	6.98	Liu et al. 1976
Daphnia magna	S	48	11.7 (10.9-12.6)[f]		Liu et al. 1983
Daphnia magna	F	48	>4.4[f]		Liu et al. 1983
Asellidae					
Hyalella azteca	S	48	6.5 (5.6-7.5)[f]	6.5	Liu et al. 1983
Insecta					
Chironomidae					
Tanytarsus dissimilis	S	48	27.0 (22.0-33.0)[f]	27.0	Liu et al. 1983
Oligochaeta					
Lumbricidae					
Lumbriculus variegatus	S	48	5.2 (4.5-6.0)[f]	12.28	Liu et al. 1983
Lumbriculus variegatus	F	48	>29.0[f]		Liu et al. 1983
Chordata					
Osteichthyes					
Centrarchidae					
Lepomis macrochirus[g]	S	96	2.3[h]	•	Pederson 1970
Lepomis macrochirus[g]	S	96	2.3[i]	•	Pederson 1970
Lepomis macrochirus[g]	S	96	2.7[j]	2.78	Pederson 1970
Lepomis macrochirus[g]	S	96	2.8[k]		Pederson 1970
Lepomis macrochirus[l]	S	96	3.4 (3.1-3.7)[m]		Liu et al. 1983
Lepomis macrochirus[l]	S	96	2.6 (2.3-2.9)[f]		Liu et al. 1983
Lepomis macrochirus[l]	F	96	2.5[f]		Liu et al. 1983

TABLE 4. (Continued)

Test Species	Test Method[b]	Test Duration (hr)	LC_{50} mg/L (CI)[c]	Genus Mean Acute Value (mg/L)[d]	Reference
Salmonidae					
Salmo gairdneri[n]	S	96	1.5 (1.2-1.8)[o]	1.34	Liu et al. 1983
Salmo gairdneri[n]	S	96	0.8 (0.7-1.0)[p]		Liu et al. 1983
Salmo gairdneri[n]	F	96	2.0[p]		Liu et al. 1983
Ictaluridae					
Ictalurus punctatus[q]	S	96	2.4 (2.0-2.7)[f]	2.81	Liu et al. 1983
Ictalurus punctatus[q]	F	96	3.3[f]		Liu et al. 1983
Cyprinidae					
Pimephales promelas[r]	F	96	2.58[s]	2.72	Smock et al. 1976
Pimephales promelas[r]	F	96	0.46 (EC_{50})[s]	*	Smock et al. 1976
Pimephales promelas[t]	S	96	2.0 (1.9-2.2)[u]		Liu et al. 1976
Pimephales promelas[t]	S	96	1.2 (0.8-1.9)[v]	*	Liu et al. 1976
Pimephales promelas[t]	S	96	2.4 (2.0-3.0)[w]	*	Liu et al. 1976
Pimephales promelas[t]	S	24	>3.2[u]	*	Liu et al. 1976
Pimephales promelas[t]	S	24	4.2[w]	*	Liu et al. 1976
Pimephales promelas[t]	S	24	3.0[w]	*	Liu et al. 1976
Pimephales promelas[z]	S	96	2.9 (2.6-3.2)[f]		Liu et al. 1983
Pimephales promelas[z]	F	96	3.7[f]		Liu et al. 1983

a. Mortality tests were designed to give LC_{50} values; immobilization tests were designed to give EC_{50} values.

b. Test methods include static (S) and measured flow-through (F).

c. Values are LC_{50}s unless indicated otherwise; numbers in () are confidence intervals.

d. Values are geometric means of the species values that meet USEPA guidelines; values not used in calculations are indicated by *.

TABLE 4. CONTINUED

Footnotes (continued)

e. Test animals were up to 12 hours old at start of test.

f. Test was performed at 20°C.

g. Test animals had a mean weight of 0.39 g.

h. Test was performed at 25°C and 60 ppm as $CaCO_3$.

i. Test was performed at 25°C and 180 ppm as $CaCO_3$.

j. Test was performed at 10°C and 60 ppm as $CaCO_3$.

k. Test was performed at 10°C and 180 ppm as $CaCO_3$.

l. Test animals had an average weight of 1.6 g and a total length of 4.0 cm at start of test.

m. Test was performed at 20°C and test solution was aerated.

n. Test animals had an average weight of 3.6 g and a total length of 6.5 cm at start of test.

o. Test was performed at 12°C and test solution was aerated.

p. Test was performed at 12°C.

q. Test animals had an average weight of 2.0 g and a total length of 5.6 cm at start of test.

r. Test animals had a mean weight of 2.5 g and an average length of 5.4 cm at start of test.

s. Test was performed at 24°C.

t. Test animals had a mean weight of 0.6 g and an average standard length of 3.3 cm at start of test.

u. Test was performed at pH 7 and 20°C.

v. Test was performed at pH 5 and 20°C.

w. Test was performed at pH 9.4 and 20°C.

x. Test animals had an average weight of 1.35 g and a total length of 4.3 cm at start of test.

TABLE 5. ACUTE STATIC TESTS OF 2,4,6-TRINITROTOLUENE TOXICITY TO *Pimephales promelas* AS AFFECTED BY pH AND ULTRAVIOLET IRRADIATION[a]

Percent Degradation	pH	LC_{50} (mg/L)[b] 24-hr	LC_{50} (mg/L)[b] 96-hr	95% Confidence Limits (96-hr)	Nonlethal Concentration
0	5	4.2	1.2	0.8-1.9	0
50	5	>3.2	2.0	-	2.4
0	7	>3.2	2.0	1.9-2.2	0
50	7	>3.2	3.0	2.1-4.2	0
100	7	48.5	44.1	40.5-48.1	37.0
0	9.4	3.0	2.4	2.0-3.0	1.0
50	9.4	>3.2	>3.2	-	3.2

a. Adapted from Liu et al. 1976.

b. Expressed in terms of the concentration of the primary chemical before irradiation.

pitching, swimming motion occurring only in response to tactile stimulation (at the highest concentrations).

Based on earlier work (e.g., Liu et al. 1976), a large study was performed by Liu et al. (1983) to determine a wide range of acute toxicity data for TNT on invertebrates and fish. Like several other studies, it evaluated toxicity or pure TNT as well as from various wastewaters associated with TNT production; only the results on pure TNT will be discussed in this report. In accordance with the USEPA (1975) guidelines for acute toxicity studies, both static and flow-through determinations of LC_{50} values were made using invertebrates (*Daphnia magna*, *Hyallela azteca*, *Tanytarsus dissimilis*, and *Lumbriculus variegatus*) and fish (*Pimephales promelas*, *Lepomis macrochirus*, *Salmo gairdneri*, and *Ictalurus punctatus*). In the static tests, fish (generally 10 per treatment) were exposed in 19-L jars containing 15 L of solution, and invertebrates (using 20 per treatment) were exposed in 250-mL beakers containing 200 mL of solution. Occasionally, test solutions were aerated if the dissolved oxygen level dropped below 60 percent during the first 48 hr or below 40 percent during the last 48 hr. TNT concentrations were not determined directly in the test containers but were calculated from the dilutions of the stock solution. The results of these evaluations are shown in Table 4 and indicate that the fish species were much more susceptible to TNT than were the invertebrates. Also, the values for the various fish species are very similar to values determined by other researchers.

The flow-through studies of Liu et al. (1983) used 19-L glass aquaria containing 15 L of solution with a delivery system providing a flow rate of 100 mL per min over a total exposure duration of 14 days. The TNT concentrations were determined every 3 to 4 days or whenever a new stock solution was used. The four fish species were exposed directly in the tanks in groups of 20 per chamber; while the invertebrates (*D. magna* and *L. variegatus*) were exposed in suspended egg cups containing 10 to 15 animals. The LC_{50} values generated in these flow-through tests are termed incipient values. An incipient LC_{50} is "the concentration above which 50 percent of the test organisms cannot survive indefinitely" (Sprague 1969, as cited in Liu et al. 1983). These values are generated by conducting time-independent acute tests in which no additional mortality occurs for at least 48 hr after the first 96 hr of exposure. The pooled incipient, 24-, 48-, and 96-hr LC_{50} values generated in these tests are given in Table 6. The incipient values are generally lower than those determined in the standard acute tests (Table 4) reported by other researchers, including Liu et al. (1983). Also the values for *D. magna* are much lower than any other reported values including fish LC_{50} values.

Bringmann and Kuehn (1980) tested several organisms for the effects of TNT using the cell multiplication inhibition test. In this assay, cultures of the bacteria *Pseudomonas putida* and the protozoa *Entosiphon sulcatum* are exposed to a series of dilution concentrations for 16 hr at 25°C, and the effects are measured by comparing turbidimetrical values expressed by the extinction of primary light of monochromatic radiation at 436 nm for a 10-mm-thick layer. Changes in the multiplication

TABLE 6. ACUTE FLOW-THROUGH TESTS FOR MORTALITY OF AQUATIC SPECIES FOLLOWING EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Test Species	LC_{50} (mg/L)				Confidence Limits for Incipient LC_{50}
	24-hr	48-hr	96-hr	Incipient[b]	
Daphnia magna	>4.4	>4.4	1.2	0.19 (192)[c]	0.12-1.0
Lumbriculus variegatus	>29.0	>29.0	>29.0	13.9 (336)	12.6-15.1
Lepomis macrochirus[d]	3.4	2.6	2.5	1.4 (312)	0.8-2.5
Salmo gairdneri[e]	2.1	2.0	2.0	1.9 (240)	1.3-3.3
Ictalurus punctatus[f]	7.4	5.6	3.3	1.6 (288)	0.9-3.0
Pimephales promelas[g]	5.9	5.9	3.7	1.5 (384)	0.9-2.5

a. Adapted from Liu et al. 1983.

b. Total hours of exposure are shown in parentheses.

c. Control mortality was 3.3 percent.

d. Test animals were 4.89 cm in average total length and 1.47 g in average weight at start of test.

e. Test animals were 7.20 cm in average total length and 4.79 g in average weight at start of test.

f. Test animals were 5.27 cm in average total length and 1.34 g in average weight at start of test.

g. Test animals were 5.69 cm in average total length and 1.38 g in average weight at start of test.

inhibition test are intended to serve as early detectors of the potential damage of water pollutants. The toxicity thresholds indicated on a regression line were >100 mg/L for the bacteria and 1.6 mg/L for the protozoa.

Another evaluation of the acute toxicity of TNT was performed by Won et al. (1976) using salt water copepods (*Tigriopus californicus*) and oyster larvae (*Crassostrea gigas*). Groups of 100 copepods and 200 oyster larvae (average age of 20 days) were incubated at 20°C for 72 and 96 hr, respectively, to various concentrations of TNT and evaluated for mortality. As shown in Table 7, concentrations greater than 5.0 mg/L resulted in a drastic increase in mortality for both species. For the oyster larvae, the 5.0- to 10.0-mg/L levels spanned a definite threshold effect level, while for the copepods, an LC_{50} of approximately 5 mg/L could be estimated. These values correspond reasonably well with established LC_{50} values for other invertebrate species (Table 4).

3.2 CHRONIC TOXICITY TO ANIMALS

Chronic toxic effects of TNT on aquatic organisms are reported in a field study and two laboratory models or studies. Putnam et al. (1981) evaluated the effects of wastewater effluents, including TNT and its degradation products, on the macroinvertebrate communities downstream from Volunteer Army Ammunition Plant (VAAP). They looked at species, numbers, diversity, and densities in two reservoir bays (one downstream from VAAP and one reference bay) by sampling artificial (Hester-Dendy plates) and naturally occurring substrates and correlating these data with sediment and water column concentrations of total munition compounds. As would be expected, effects on these parameters generally increased as samples were taken closer to VAAP but were noticeable as far as one mile downstream. They found no effects on macroinvertebrates at total munition concentrations of 25 μg/L, definite effects at average concentrations of 500 to 600 μg/L (with a maximum value recorded of over 2,000 μg/L), and a suggested minimal effect at concentrations of 50 to 100 μg/L. However, a direct correlation with TNT concentrations alone was not established.

One laboratory study of the effects of TNT on aquatic organisms was based on a microcosm approach that contained the zooplankton *Daphnia magna*, the benthic oligochaete *Lumbriculus variegatus*, and the alga *Selenastrum capricornutum* (Bailey 1982). An interactive community of these species was set up in aerated 3-L jars containing 10,000 to 15,000 algal cells/mL, 15 daphnids, and 30 worms for 21 days. Concentrations of TNT solutions were added to the systems to produce duplicate exposures of all concentrations (0, 0.6, 1.0, 5.6, 10.0, and 24.0 mg/L). The concentrations were sampled at least daily and analyzed by high-pressure liquid chromatography to detect any degradation products or reduction of TNT concentrations. Observations included effects on numbers and conditions of invertebrates at the end of 21 days and on reproductive success of daphnids during exposure. If reproduction increased the daphnid population above 100 young, then the excess young were culled so as to prevent mortality from overpopulation. The TNT levels

TABLE 7. TOXICITY OF 2,4,6-TRINITROTOLUENE FOLLOWING 3-DAY EXPOSURE OF *Tigriopus californicus* AND 4-DAY EXPOSURE OF *Crassostrea gigas*[a]

TNT (mg/L)	Mortality	
	T. californicus	*C. gigas*
100	100/100[b]	200/200
50	100/100	200/200
25	100/100	200/200
10	100/100	163/200
5	44/100	0/200
2.5	18/100	0/200
1.0	0/100	0/200
0	0/100	0/200

a. Adapted from Won et al. 1976.

b. Number of dead/number exposed.

in the jars did decrease with time, in proportion to the initial concentrations. The total numbers of daphnids were reduced at all concentrations. Based on the LC_{50} values, reductions at levels below 5.6 mg/L were attributed by the author to insufficient food due to reduced algal populations or chronic-type effects of low-level exposures. The total number of worms was reduced at concentrations as low as 5.6 mg/L; the size of these worms was also affected. Bailey compared these data with acute LC_{50} values measured in his laboratory (see data of Liu et al. in Tables 5 and 6) and found that the toxic concentrations were similar. Although this was a 21-day test, it could not be considered a definitive evaluation of the chronic effects of TNT on invertebrates due to its static design, exclusion of observations for reproductive effects, and lack of stable TNT concentrations.

Bailey et al. (1985) further tested the chronic effects of TNT on *D. magna*, using a 28-day exposure period. The tests were conducted under flow-through conditions in 400-mL beakers placed in 80-L aquaria containing 28 L of water arranged so that water could enter the beakers through mesh-covered holes. At the start of the test, young daphnids were placed in the beakers. Each day the beakers were observed for mortality of adult daphnids and production of young, which were removed and counted. After 28 days, the remaining daphnids were removed from the beaker and measured using an ocular micrometer. Observations in this test included mortality at weekly intervals (test days 7, 14, 21, and 28); total reproduction at weekly intervals; young produced per female at weekly intervals; young produced per female per reproductive day; length at day 28; and days until first young produced. The data from daphnid exposure to TNT indicated no significant effects on survival at the tested concentrations (0.03, 0.08, 0.24, 0.48, and 1.03 mg/L). The majority of reproductive parameters measured also indicated no significant dose-response effects of TNT at the tested doses. Significant decreases in young produced at the highest dose (1.03 mg/L) occurred on days 14 and 21, but by day 28 cumulative reproduction exceeded that of controls. The authors felt this pattern indicated a transitory effect of TNT on reproduction but also cautioned that average daphnid survival in the real world would be less than 28 days; thus, the impaired reproduction could have biological significance (Bailey et al. 1985).

A laboratory evaluation of the chronic toxicity of TNT on fish was also reported by Bailey et al. (1985). They investigated the effects of TNT in early life stage experiments using rainbow trout (*Salmo gairdneri*), channel catfish (*Ictalurus punctatus*), and several generations of fathead minnows (*Pimephales promelas*).

In the early life stage tests, flow-through conditions were employed with water supply and toxicant supply reservoirs connected to a mixing chamber to provide proper exposure levels and a minimum flow of four tank volumes per day. Tests were performed in 19-L tanks containing 15 L of solution with six exposure levels (in duplicate). Chemical concentrations were determined prior to testing and at weekly intervals thereafter. Nominal temperatures of 12°C for trout and 25°C for fatheads and catfish and a photoperiod of 16 hrs of light were used. In the trout study, 60 eggs per duplicate tank at each exposure level

were fertilized in the presence of TNT. Two series of studies were run with an exposure period of 60 days post-hatch. For the fathead minnows, tests were initiated with 30 embryos per duplicate tank at each exposure level, and embryos were observed for 30 days post-hatch. The catfish presented a problem, because the eggs could not be individually separated after fertilization. Therefore, egg masses of similar weights were distributed to each tank, and a 30-day post-hatch treatment period was used. After treatment, total length was determined for each test organism and, in addition, the trout were weighed. Results from these tests are given in Table 8. Generally, the results for egg hatching or fry survival indicate that levels up to and including 1.35 mg/L had no effect on catfish, levels of 0.93 mg/L significantly reduced trout survival and growth, and levels of 0.84 mg/L affected fry survival in fathead minnows. Results from the fathead minnow and channel catfish early life history tests are questionable due to the problem with survival of fry. Because of high variability and problems with survival of trout fry in controls, an additional 60-day study was performed using trout. The results of this study (Table 8, test 2) indicate that egg hatch was not affected, but fry survival was significantly reduced at levels of 0.24 to 1.69 mg/L. The evaluations of the effects of TNT on total length of the trout fry (Table 8, series B) showed decreases at levels of ≥0.49 mg/L in trout (≥0.50 mg/L in second test). Data for the other species did not show growth effects of TNT.

Bailey et al. (1985) also studied effects of chronic exposure to TNT of fathead minnows over several generations. The general test procedure was the same as in the multi-species fish test described above. The tests with the fathead involved hatching fry from at least 40 eggs at each concentration, observing the fry for 90 days, rearing the fry into mature adults, breeding 4 pairs of mature adults at each dose, observing the spawning success of these breeders, and rearing the fry from the successful spawning efforts. Many indices of toxic effects were evaluated in this test, including evaluations of the parent generation (F0) and their offspring (F1) (Table 9). The results of the TNT exposure on the egg hatching success, fry survival, and fry growth in the F0 generation (Table 10) indicate significant effects on egg hatch at 1.21 mg/L, on fry survival at 0.25 to 1.21 mg/L, and early effects (day 30) on fry growth. Data on reproductive effects of chronic exposure to TNT indicate a wide range of damage with almost all parameters affected adversely at concentrations of 0.04 to 1.21 (Table 11). Effects that were not statistically significant were generally still lower than control values. The least affected parameter was number of eggs per spawn which indicated to the authors that the overall effect on reproduction was due to reductions in the survival of the breeding pairs and frequency of spawns per pair (Bailey et al. 1985). Statistically significant effects of TNT on the F1 generation included lower numbers of eggs hatched at all concentrations, increased numbers of deformed fry at 0.10 to 0.56 mg/L, decreased fry survival the first 30 days of exposure at 0.04 to 0.25 mg/L, and reduced length and weight of fry surviving to 60 days at 0.04 to 0.25 mg/L (Table 12). This general pattern of effects on reproduction in both generations is also reflected in total survivability data and productivity indices (Table 13). The summation of the chronic study on fathead minnows by Bailey et al. (1985)

TABLE 8. EGG HATCHING SUCCESS AND FRY SURVIVAL IN THREE FISH SPECIES AFTER CHRONIC EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Species	TNT Level (mg/L)	Test Series	Eggs		Fry Alive at End	Avg. Fry Length (cm)
			No. Exposed	No. Hatched		
Channel Catfish	0	A	34	11	4	
		B	38	10	4	
	0.11	A	40	20	11	
		B	54	33	17	
	0.15	A	48	29	20	
		B	43	14	6	
	0.30	A	59	36	21	
		B	60	31	27	
	0.66	A	57	35	32	
		B	51	33	28	
	1.35	A	67	37	23	
		B	59	24	19	
Fathead Minnow	0	A	60	60	32	1.03
		B	60	57	27	1.05
	0.07	A	60	57	36	1.08
		B	60	57	35	1.08
	0.10	A	60	60	36	1.12
		B	60	59	36	1.14
	0.16	A	60	53	40	1.03
		B	60	58	37	1.08
	0.42	A	60	41	26	1.12
		B	60	54	25	1.10
	0.84	A	60	55	15	1.14
		B	60	57	21	0.93

TABLE 8. CONTINUED

Species	TNT Level (mg/L)	Test Series	Eggs		Fry Alive at End	Avg. Fry Length (cm)
			No. Exposed	No. Hatched		
Rainbow Trout						
Test 1	0	A	61	61	37	2.43
		B	63	63	37	2.42
	0.07	A	60	56	3[b]	2.44
		B	60	56	10[b]	2.27
	0.12	A	60	59	30	2.33
		B	61	61	42	2.21[b]
	0.21	A	61	44[b]	12	2.54
		B	60	49[b]	21	2.45
	0.49	A	60	59	26	2.21[b]
		B	60	60	41	2.05[b]
	0.93	A	62	44[b]	14[b]	1.93[b]
		B	64	37[b]	8[b]	1.96[b]
Test 2	0	A	60	53	17[c]	4.25
		B	60	52	49	3.69
	0.02	A	60	51	44	3.67[b]
		B	60	55	44	3.61
	0.04	A	60	55	42	3.68[b]
		B	61	49	36	3.89
	0.13	A	60	52	40	3.55[b]
		B	60	50	44	3.57
	0.24	A	60	52	36[b]	3.62[b]
		B	61	51	37[b]	3.65
	0.50	A	60	51	38[b]	3.25[b]
		B	61	52	36[b]	3.37[b]
	0.87	A	60	55	35[b]	3.11[b]
		B	60	48	28[b]	3.03[b]
	1.69	A	62	56	3[b]	2.18[b]
		B	60	56	2[b]	2.38[b]

a. Adapted from Bailey et al. 1985.

b. Statistically significant at $p < 0.05$.

c. When tank was flooded, 29 fry were lost; later calculations based on control values were corrected based on projections of control values.

TABLE 9. TESTS ANALYZED TO INDICATE TOXIC EFFECTS IN FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO 2,4,6-TRINITROTOLUENE[a]

F_0 Observations	F_1 Observations	Global Observations
Egg survival	Egg survival	F_0 90-d Cumulative fry survival
30-d Fry survival	30-d Fry survival	F_0 178-d Cumulative fry survival
60-d Fry survival	60-d Fry survival	F_0 60-d Biomass
90-d Fry survival	Deformities	F_1 60-d Cumulative fry survival
120-d Fry survival	30-d Length	F_1 60-d Standing crop
150-d Fry survival	30-d Weight	Total survivability index
178-d Fry survival	60-d Length	Total productivity index
30-d Length	60-d Weight	
60-d Length		
90-d Length		
Fry deformities		
Breeding pair survival		
Spawns per pair		
Eggs per spawn		
Eggs per pair per day		
Eggs per pair		

a. Adapted from Bailey et al. 1985.

TABLE 10. EGG HATCHING SUCCESS, FRY SURVIVAL, AND FRY GROWTH IN FATHEAD MINNOWS (*Pimephales promelas*) AFTER CHRONIC EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Average TNT Concentration (mg/L)	Test Series	Eggs		No. Fry Deformed	Fry Survival						Fry Growth		
					Days Exposed						Days Exposed		
		No. Exposed	No. Hatched		30	60	90	120	150	178	30	60	90
0	A	100	96	3	87	86	81	81	80	80	1.88	2.57	3.15
	B	110	90	2	74	73	72	72	72	71	1.80	2.98	3.90
0.04	A	100	100	3	89	89	83	79	77	77	1.89	2.54	3.14
	B	110	97	0	72	71	69	69	69	67	1.99	2.92	3.67
0.10	A	100	96	1	89	85	84	81	80	78	1.93	2.49	3.10
	B	110	78	1	54	54	53	53	55	55	2.01	3.14	4.09
0.25	A	100	99	2	77[b]	76[b]	73[c]	69[b]	67[b]	64[b]	1.86	2.63	3.11
	B	110	89	0	61[c]	61[c]	59[c]	59[c]	59[c]	59[c]	2.00	3.01	4.20
0.56	A	100	96	4	73[b]	68[b]	65[b]	55[b]	52[b]	48[b]	1.39[b]	2.40	3.12
	B	110	82	1	57[c]	56[c]	55[c]	54[c]	53[c]	47[c]	1.94	3.10	3.85
1.21	A	100	99[c]	5	43[b]	40[b]	33[b]	25[b]	22[b]	22[b]	1.70[b]	2.61	3.22
	B	110	68[b]	0	18[b]	15[b]	13[b]	10[b]	9[b]	7[b]	2.05	3.51	4.58

a. Adapted from Bailey et al. 1985

b. Statistically significant at $p < 0.05$.

c. Statistically significant at $p < 0.05$, if the series are pooled.

TABLE 11. REPRODUCTIVE PARAMETERS IN FATHEAD MINNOWS (*Pimephales promelas*) AFTER CHRONIC EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Average TNT Concentration (mg/L)	Test Series	Spawning Pair Survival (day)	No. of Spawns per Pair	Eggs per Pair	Eggs per Spawn	Eggs per Pair per Day
0	A	113	16	2948	184.2	26.1
	B	201	29	5054	174.3	25.1
0.04	A	82[b]	12	1954[b]	162.8	23.8
	B	56[c]	8[c]	1255[b]	156.9	22.4
0.10	A	104[b]	5[c]	898[c]	179.6	8.6[b]
	B	81[c]	5[c]	444[c]	88.8	5.5[b]
0.25	A	84	7[b]	1450[b]	207.1	17.3
	B	145	12[b]	1839[b]	153.2	12.7
0.56	A	77[b]	3[c]	209[c]	69.7	2.7[c]
	B	85[c]	1[c]	120[c]	120.0	1.4[c]
1.21	A	81[b]	0[c]	0[c]	0[c]	0[c]
	B	43[c]	0[c]	0[c]	0[c]	0[c]

a. Adapted from Bailey et al. 1985.

b. Statistically significant at $p < 0.05$, if the series are pooled.

c. Statistically significant at $p < 0.05$.

TABLE 12. EFFECT OF CHRONIC EXPOSURE TO 2,4,6-TRINITROTOLUENE ON F1 FATHEAD MINNOWS (*Pimephales promelas*)[a]

Average TNT Concentration (mg/L)	Test Series	Eggs		No. of Fry Deformed	Fry at 30 days				Fry at 60 days			
		No. Exposed	No. Hatched		No. Transferred	No. Survived	Length (cm)	Weight (g)	No. Transferred	No. Survived	Length (cm)	Weight (g)
0	A	1150	777	26	79	71	1.91	0.050	91	59	2.87	0.174
	B	2000	1432	53	78	76	1.82	0.050	73	61	2.72	0.182
0.04	A	850	583[b]	25	77	52[c]	1.88	0.076	82	66	2.58[c]	0.121[c]
	B	950	388[c]	19	38	30[c]	1.88	0.050	34	28	2.90[b]	0.227[b]
0.10	A	450	287[b]	36[b]	36	34[b]	1.70[c]	0.041	45	34	2.46[b]	0.097[c]
	B	345	183[c]	6[b]	31	12[c]	1.81[b]	0.056	38	34	2.68[b]	0.147[c]
0.25	A	750	497[b]	82[c]	62	60[b]	1.93	0.064	71	58	2.73[c]	0.161[b]
	B	848	490[b]	57[c]	73	33[c]	2.01	0.081	66	60	2.66[b]	0.162[c]
0.56	A	250	127[b]	14[c]	0	-	-	-	0	-	-	-
	B	150	52[c]	2[b]	0	-	-	-	0	-	-	-
1.21	A	0	0	0	0	-	-	-	0	-	-	-
	B	0	0	0	0	-	-	-	0	-	-	-

a. Adapted from Bailey et al. 1985.

b. Statistically significant at $p < 0.05$, if series are pooled.

c. Statistically significant at $p < 0.05$.

TABLE 13. TOTAL SURVIVABILITY AND PRODUCTIVITY INDICES IN FATHEAD MINNOWS (*Pimephales promelas*) AFTER CHRONIC EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Average TNT Concentration (mg/L)	Test Series	Total Survivability[b]	Total Productivity[c]
0	A	999	167
	B	1880	345
0.04	A	795	95
	B	244	55
0.10	A	318	31
	B	102	15
0.25	A	419	64
	B	458	75
0.56	A	45	4
	B	0	0
1.21	A	0	0
	B	0	0

a. Adapted from Bailey et al. 1985.

b. Total survivability is defined as the product of the cumulative F_0 survival to 180 days, the average number of eggs per female, and the cumulative F_1 survival after 60 days of exposure.

c. Total productivity is defined as the product of the total survivability index and the average weight of the F_1 generation after 60 days of exposure.

indicated that TNT had significant chronic effects at all concentrations tested (0.04 to 1.21 mg/L).

3.3 TOXICITY TO PLANTS

The majority of information on the toxicity of TNT to aquatic plants deals with acute exposures of algal species. However, one study did report the impact of TNT on the vascular plant species *Lemna perpusilla* (Schott and Worthley 1974). This species of duckweed was exposed to graded concentrations of TNT for 11 days at two pH levels (6.3 and 8.5), and observations were made of deaths or any reduction in frond growth rate. As shown in Table 14, a level of 1.0 ppm resulted in reduced growth, and a level of 5.0 ppm resulted in plant death. The pH of the test solution did not appear to affect the toxicity. These levels were also compared to the effect levels of a widely used herbicide, 2,4-dichlorophenoxyacetic acid, and were only an order of magnitude higher at pH of 6.3.

Short-term tests of the toxicity of TNT to algal species were evaluated using a variety of growth measurements. Fitzgerald et al. (1952) exposed cultures of *Microcystis aeruginosa* grown in 125-mL flasks at 22°C to TNT for 24 hrs and found that a level of 8 ppm resulted in 100 percent mortality. Gring (1971) exposed cultures of *Chlamydomonas reinhardi* to TNT and stated that a level of 3.0 µg/mL was "quite toxic." Won et al. (1976) exposed cultures of *Selenastrum capricornutum* grown in 125-mL flasks for 7 days to concentrations of TNT and found that a level of 2.5 mg/L reduced growth (mean dry weight) and that a level of 10.0 mg/L resulted in death. The 2.5-mg/L level also resulted in chlorosis and cells that were ballooned and extensively granulated. Bringmann and Kuehn (1980) tested *Scenedesmus quadricauda* colonies for the effects of TNT using the cell multiplication inhibition test. In this assay, colonies are exposed to a series of TNT dilution concentrations for 16 hr at 27°C, and the effects are measured by comparing turbidimetric values expressed by the extinction of primary light of monochromatic radiation at 578 nm for a 10-mm-thick layer. The toxicity threshold generated was 1.6 mg/L.

In longer term studies, Smock et al. (1976) tested cultures of *M. aeruginosa* and *S. capricornutum* grown in 500-mL flasks for growth effects over 15 to 17 days of exposure to TNT. Effects on growth of *S. capricornutum* were not seen at levels up to 3 mg/L, and only an initial decrease in growth (as compared with controls) occurred at 5 mg/L and disappeared after 7 days. However, a permanent and significant decrease in growth of *S. capricornutum* occurred at levels of 7 and 9 mg/L. In the test of *M. aeruginosa*, no effects on growth occurred at levels up to 15 mg/L, temporary decreases occurred at 25 mg/L, and permanent decreases occurred at 50 mg/L. In tests of both species, TNT concentrations were found to decrease by at least the seventh day, indicating that decomposition to other compounds had occurred, which might explain the growth increase after 7 days at the intermediate dose concentrations. Anatomical changes in the algal cells included vacuole and sheath production which was proportional to the TNT concentrations.

TABLE 14. RESPONSE OF *Lemna perpusilla* COLONIES GROWN IN AQUEOUS MEDIA FOLLOWING 11-DAY EXPOSURE TO 2,4,6-TRINITROTOULENE[a]

TNT (ppm)	pH	Average No. of Fronds[b]	Results[c]
50.0	6.3	0	Death
10.0	6.3	0	Death
5.0	6.3	0	Death
1.0	6.3	32	Growth depressed
0.5	6.3	65	No effect
0.1	6.3	73	No effect
0.01	6.3	71	No effect
0	6.3	71	Control
50.0	8.5	0	Death
10.0	8.5	0	Death
5.0	8.5	0	Death
1.0	8.5	7	Growth depressed
0.1	8.5	35	No effect
0	8.5	33	Control

a. Adapted from Schott and Worthley 1974.

b. Each test started with 2 fronds on day 0.

c. Colonies growing at least 10 percent slower than controls were considered to be depressed.

Liu et al. (1983) studied the effects of TNT on the species *S. capricornutum* (green algae), *M. aeruginosa* (bluegreen algae), *Anabaena flos-aquae (bluegreen algae), and* Navicula pelliculosa (diatom) using the USEPA (1971) bottle assay technique. Effects were detected by measuring cell concentration, biomass, and chlorophyll *a* concentration following exposure to a series of TNT concentrations for 14 days. The tests were conducted in 500-mL flasks with 100 mL of test solution at 24°C under "cool-white" fluorescent light. However, problems in the test eventually led to using only the cell concentration to evaluate the impact of the TNT exposure. The predominant effect of TNT was population growth inhibition. The lowest concentrations at which this effect occurred significantly were 4.1 mg/L for *S. capricornutum* and *M. aeruginosa*, 8.2 mg/L for *A. flos-aquae*, and 18 mg/L for *N. pelliculosa*. However, again problems were encountered with the algal bottle assay procedures. The light required for algal growth also photolyzed the TNT solution (e.g., dropping an initial concentration of 20.8 mg/L to 5.3 mg/L by test day 14 and creating a brownish-pink color indicating photolysis). Thus, Liu et al. concluded that the toxicity estimates were unreliable; effects could not be solely attributed to the TNT compound because of the possible formation of degradation compounds. They also concluded that the bottle test is inappropriate for evaluating the toxicity of a photosensitive compound such as TNT, but unfortunately a satisfactory replacement technique is not available.

A microcosm approach to the longer-term exposure to TNT using the zooplankton *Daphnia magna*, the benthic oligochaete *Lumbriculus variegatus*, and the alga *S. capricornutum* was performed by Bailey (1982). An interactive community of these species was attained in aerated 3-L jars containing 10,000 to 15,000 algal cells/mL, 15 daphnids, and 30 worms for 21 days. TNT solutions were added to the systems to produce duplicate exposures of all concentrations (0, 0.6, 1.0, 5.6, 10.0, and 24.0 mg/L). The concentrations were sampled at least daily and analyzed with high-pressure liquid chromatography to detect any degradation or reduction of TNT exposures. Observations of algal densities were made every 3 days during the 21-day exposure. The TNT levels in the jars decreased with time in proportion to the initial concentrations. The algal densities at TNT levels of 5.6 mg/L and higher were decreased during the test period. Densities at 1.0 mg/L initially declined but gradually increased by the end of the test period. The two tests at 0.6 mg/L differed quite sharply in their response, with the result that the mean value showed no difference from the control values. Bailey felt that this might indicate that the threshold level is very close to the 0.6 mg/L concentration. Although this was a 21-day test, due to its static design and lack of stable TNT concentrations, it could not be considered an extensive evaluation of the chronic effects of TNT on algae.

Putnam et al. (1981) evaluated the effects of wastewater effluents, including TNT and its degradation products, on the periphyton communities downstream from VAAP. They looked at cell densities, biomass estimates, and community structure in two reservoir bays (one downstream from VAAP and one reference bay) by using artificial substrates and correlating the data with sediment and water column concentrations of total munition compounds. They found biomass and population size varied

inversely with VAAP waste concentrations. The effects included decreased population size near VAAP (27 organisms/mm^2 vs 37,367 organisms/mm^2) and lower numbers of species (22 vs 52) at station 2 (0.25 km down bay) and station 6 (1.25 km down bay), respectively. The population density was significantly different at stations 1 to 5 in comparison with stations 6 to 12. The overall pattern of effects on periphyton indicated toxicity at the bayhead followed by biological stimulation downstream. Putnam et al. found no effects on periphyton at total munition concentrations of 25 $\mu g/L$ but definite effects at average total munition concentrations of 575 $\mu g/L$ (with a peak of 2,370 $\mu g/L$). However, due to the variety of compounds present, no direct correlation with TNT concentrations alone was determined.

3.4 BIOACCUMULATION

The bioconcentration potential of TNT was evaluated by Liu et al. (1983) using ^{14}C-labelled TNT. The tests were performed for 96 hr under static exposure conditions at 20°C with the amount of ^{14}C-TNT measured at the end of the exposure. Three *Lepomis macrochirus* were exposed in 10-L containers, 50 *Lumbriculus variegatus* were exposed in 2-L containers, 100 *Dapnnia magna* were exposed in 2-L containers, and 10,000 algal cells of *Selenastrum capricornutum* were exposed in 100 mL of solution in a 500-mL beaker. The TNT was dissolved in dimethyl sulfoxide (DMSO) with 0.5 mg of TNT and 0.2 μCi of radioactivity in the test solution. Following the 96-hr exposure period, the invertebrates and algae were filtered and weighed, while tissue samples (muscle and viscera) were removed from the fish and weighed. The samples were then solubilized in Unisol, and 1 mL was counted for 10 minutes in a liquid scintillation counter equipped with an automatic background subtractor. The 4-day bioconcentration factor (BCF) was calculated by dividing the average amount of radioactivity found in the biological samples by the amount in the test water. For the intact organisms, the BCFs ranged from 200 to 450, with the highest BCF occurring in the algae(Table 15). Based on tissue samples in *L. macrochirus*, the BCFs were 338 for viscera and 9.5 for muscle. The large difference in these values was attributed to the metabolism of TNT in the liver included in the viscera sample. The BCF values obtained in this study are not very useful because they represent only one point on the uptake curve (rather than a steady-state value) and no difference was made between the radioactivity of TNT and its metabolic products. Recognizing this shortcoming, Liu et al. (1983) also calculated the octanol-water partition coefficient using the methods of Hansch et al. (1973) and Leo (1976) as an index of the probability of bioconcentration. A value of 2.03 was calculated for Log P, which does not indicate a high likelihood of bioconcentration because chemicals with values below 3.0 are not considered to bioconcentrate extensively. Taking this further, Liu et al. used the direct relationship indicated between log P and the steady-state BCF developed by Veith and coworkers (1978). Based on their equation, a steady-state BCF for TNT was estimated to be 20.5, which indicates only a low potential for bioconcentration based on the guidelines of Cairns and Dickson (1978).

TABLE 15. CONCENTRATION OF 2,4,6-TRINITROTOLUENE IN SELECTED AQUATIC ORGANISMS AFTER 96 hr OF EXPOSURE[a]

Organism	Average Amount of Radioactivity Recovered[b]		
	Water[c]	Tissue	4-Day BCF[d]
Selenastrum capricornutum	356	161,162	453.0
Daphnia magna	519	108,643	209.0
Lumbriculus variegatus	450	91,092	202.0
Lepomis macrochirus			
Viscera	453	153,017	338.0
Muscle	453	4,301	9.5

a. Adapted from Liu et al. 1983.

b. Disintegrations per minute per gram.

c. At begining of test.

d. BCF = bioconcentration factor is the average amount of radioactivity in tissue/the average amount of radioactivity in water.

The bioconcentration and distribution of TNT by four species was investigated by Xu and Chen (1983) using an aquatic microcosm. *Tilapia mossambica*, *Belbamya purificata*, *Pislia stratiotes* and *Anlirrhinum majus* were exposed to ^{14}C-labelled TNT, and the resulting calculated bioconcentration values (based on dry wt.) were 210, 171, 1165-1415, and 1623-2030, respectively. In fish, the highest concentrations of radioactivity were found in the gills, with progressively less in the viscera, muscle, and bone. The amount of radioactivity contained in the microcosm indicated a decline in TNT concentration from 10 to 3.49 ppm 30 days after the exposures.

3.5 SUMMARY

Studies of the acute toxicity of TNT to aquatic organisms indicate that LC_{50} values range from 5.2 to 27.0 mg/L for invertebrates in 48-hr static tests and from 0.8 to 3.7 mg/L for fish in 96-hr static tests. In 96-hr flow-through tests, the LC_{50} values were 2.0 to 3.3 mg/L for fish. Evaluations of factors that might affect the acute toxicity of TNT indicate that water hardness is not a significant factor, but toxicity decreases slightly with increasing pH, temperature, and photodegradation. Using the incipient LC_{50}, a more sensitive measure of toxicity, values of 0.19 to 13.9 mg/L were found for invertebrates, and values of 1.4 to 1.9 mg/L were found for fish species.

Data on the chronic effects of TNT included an environmental survey of an Army Ammunition Plant which produced TNT as well as several laboratory studies. Wastewater effluent produced effects on macroinvertebrate species numbers, diversity, and density in a reservoir bay downstream from Volunteer AAP. Total munition concentrations (including TNT) of 0.025 mg/L produced no effects on macroinvertebrate communities, but definite effects were noted at 0.150 to 0.600 mg/L with a suggested minimal effect level of 0.050 to 0.100 mg/L. No direct correlation between TNT and macroinvertebrate population dynamics could be made. A laboratory microcosm study using daphnid zooplankton, worms, and algae exposed statically to TNT for 21 days produced significant reductions in total numbers of daphnids and worms at levels of $\geq$5.6 mg/L. A 28-day study of effects on daphnids exposed in flow-through conditions to TNT levels of 0.03 to 1.03 mg/L produced no significant effects on survival or reproduction. Some transitory effects (at test days 14 and 21) in lower numbers of young produced per female at 1.03 mg/L might be biologically significant because of the shorter lifespan of daphnids in the real world environment. Chronic studies of early life stages of three fish species exposed to TNT in flow-through conditions for 30 to 60 days (post-hatch) measured a variety of toxic impacts. In 60-day studies of trout eggs and fry, levels of 0.24 to 1.69 mg/L significantly reduced fry survival, while levels of 0.40 to 0.50 mg/L significantly reduced total length and weight of fry. A three-generation study of fathead minnows conducted under flow-through conditions indicated significant effects based on reproductive and growth indices. Concentrations of 0.04 to 1.21 mg/L TNT reduced fry growth, lowered hatching rates, lowered survival, and affected the later generations more severely than the parent generation.

Toxic effects of TNT on plants were shown in studies on algae and duckweed species. A level of 1.0 ppm reduced growth, and a level of 5.0 ppm produced death in duckweed after 11 days of exposure. Studies in several algal species indicated concentrations of 2.5 to 9.0 mg/L reduced growth and concentrations of 8 to 10 mg/L resulted in death. In a 21-day microcosm study, concentrations of 5.6 mg/L significantly reduced algal growth, while concentrations of 0.6 to 1.0 mg/L indicated transitory or initial effects on growth. The 0.6-mg/L concentration may be close to the threshold level for the alga used in this study. However, actual TNT concentrations were altered by photolysis, and effects may also be due to break-down products.

Bioconcentration Factors (BCFs) calculated from data for TNT were 9.5 and 338 for muscle and viscera, respectively, of bluegills. These values were point estimates from the uptake curve and are not highly reliable. Other BCF estimates included 210 to 2030 for four, non-native fish species and a calculated BCF value of 20.5 (based on the octanol-water partition coefficient). The general consensus is that TNT will not highly bioconcentrate according to guidelines of Cairns and Dickson (1978).

4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS

4.1 PHARMACOKINECTICS

Trinitrotoluene is absorbed by humans and test animals through the skin, by ingestion, and by inhalation (Voegtlin et al. 1921-22; Zakhari et al. 1978). Early studies indicated that TNT applied to the shaved backs of pigs (2 g in a gauze bandage) or to the palms of two humans (500 mg in rubber gloves) resulted in the presence of 2,6-dinitro-4-aminotoluene (a metabolite of TNT) in the urine over the following 15 hr to 8 days (Neal et al. 1944, as cited in Zakhari et al. 1978). Absorption of TNT from the gastrointestinal (GI) tract of humans was also reported by Horecker and Snyder (1944, as cited in Zakhari et al. 1978), who gave two humans daily doses of 1 mg/kg for 4 days. Again the presence of 2,6-dinitro-4-aminotoluene in the urine, representing 3 percent of the TNT dose, was used to indicate absorption. Von Oettingen et al. (1944, as cited in Zakhari et al. 1978) demonstrated that 75 percent of a daily TNT dose of 25 to 50 mg/kg blown into the respiratory tract of dogs was absorbed. More recent studies (Hodgson et al. 1977; Ellis et al. 1980; El-Hawari et al. 1981) using ^{14}C-labelled TNT confirm these earlier findings and indicate that the amount absorbed varies among different routes and test species (Tables 16, 17, 18). Absorption was slower by the dermal route than by the intratracheal and oral routes (El-Hawari et al. 1981). Intratracheal administration of ^{14}C-TNT in rats produced rapid, uniform absorption with high urine, bile, GI tract, and blood levels after 4 hr (Table 18). Radioactivity levels following intratracheal administration were higher in all organs except in the GI tract than levels following oral administration.

Following oral administration, ^{14}C-TNT is distributed at low levels throughout the body (Ellis et al. 1980) and is found at highest levels in the GI tract, with less than 1 percent found in liver, kidneys, and blood (Table 16). Following dermal administration, muscle tissue contained higher levels of ^{14}C-TNT than after oral administration, but the levels in the liver and kidneys were slightly lower (El-Hawari et al. 1981). With intratracheal administration, similar tissues were involved (Table 18), but ^{14}C-TNT generally was found at higher concentrations in all organs with less (13 to 20 percent) excreted in urine. Also, more radioactivity was found in bile and much less in the GI tract than after oral administration. At 24 hr, regardless of route, the majority of radioactivity was found in the urine and feces in all species. Although experiments were not extended beyond 24 hr, there is an indication that retention of ^{14}C-TNT is not extensive (El-Hawari et al. 1981). This conclusion is supported by an abstracted report of a Russian kinetic study which showed no accumulative effect in organs of rats after intraperitoneal (IP) administration of 0.2 to 4 mg/kg ^{14}C-TNT (Dymova et al. 1984).

As indicated by the distribution data, the primary route of excretion is the urine. Hodgson et al. (1977) reported that 45 to 70 percent of the radioactivity of an orally administered ^{14}C-TNT dose appeared in the urine of four species within 24 hr (Table 16), with females

TABLE 16. DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 hr AFTER ORAL ADMINISTRATION OF ^{14}C-2,4,6-TRINITROTOLUENE[a]

Tissue or Sample	Percent of Administered Dose							
	Rats[b]		Mice		Rabbits[c]		Dogs[d]	
	Males	Females	Males[e]	Females[f]	Males	Females	Males	Females
GI Tract and Contents	29.7	33.9	12.4	7.4	7.5	10.8	9.9	4.3
Feces	8.1	2.1	42.6	18.2	1.8	1.8	5.4	16.7
Urine	52.7	64.5	44.7	60.5	66.3	78.9	59.1	60.0
Blood[g]	0.2	0.3	0.5	<0.1	0.3	0.3	1.2	1.9
Spleen	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	0.2
Liver	0.4	0.4	0.7	0.5	1.0	0.9	2.2	1.8
Kidney	0.2	0.2	0.4	<0.1	0.1	0.1	0.1	0.2
Brain	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Lung	<0.1	<0.1	<0.1	<0.1	0.2	0.2	0.1	0.2
Muscle[h]	<0.1	<0.1	<0.1	<0.1	1.0	1.8	1.4	2.2
Recovered	91.3	103	101.4	86.8	78.1	94.7	79.6	87.5

a. Adapted from Hodgson et al. 1977.

b. Based on dose of 100 mg/kg and a mean of four rats.

c. Based on dose of 5 μg/kg and a mean of three rabbits.

d. Based on dose of 5 μg/kg and a mean of three dogs.

e. Based on dose of 100 mg/kg and a mean of seven mice.

f. Based on dose of 100 mg/kg and a mean of eight mice.

g. Based on 7 percent of body weight.

h. Based on 40 percent of body weight.

TABLE 17. DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 24 hr AFTER DERMAL ADMINISTRATION OF ^{14}C-2,4,6-TRINITROTOLUENE[a]

Tissue or Sample	Percent of Administered Dose						
	Rats[b]		Mice[c]	Rabbits[d]		Dogs[e]	
	Males	Females	Males	5 mg/kg	50 mg/kg	5 mg/kg	50 mg/kg
GI Tract and Contents	3.1	6.4	3.6	5.8	5.8	1.7	1.7
Feces	1.3	2.5	14.2	7.8	2.8	1.7	0.8
Urine	17.4	14.6	22.7	52.9	47.2	11.7	11.8
Blood[f]	0.2	0.3	0.2	0.3	0.3	0.3	0.4
Spleen	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Liver	0.2	0.2	0.3	0.6	0.7	0.5	0.7
Kidney	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Brain	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Lung	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1
Muscle[g]	0.5	0.9	0.6	0.9	0.5	0.7	0.4
Recovered[h]	22.8	24.9	41.7	68.3	56.9	16.8	15.9

a. Adapted from El-Hawari et al. 1981.

b. Based on mean of six rats at 50 mg/kg.

c. Based on mean of six mice at 50 mg/kg.

d. Based on mean of four males at 5 mg/kg or two males at 50 mg/kg.

e. Based on mean of three males at 5 mg/kg or one male at 50 mg/kg.

f. Based on 7 percent of body weight.

g. Based on 40 percent of body weight.

h. Fat, bile, and skin are not included in recovery.

TABLE 18. DISTRIBUTION AND EXCRETION OF RADIOACTIVITY 4 hr AFTER ORAL AND INTRATRACHEAL ADMINISTRATION OF ^{14}C-2,4,6-TRINITROTOLUENE TO RATS[a]

Tissue or Sample	Percent of Administered Dose[b]			
	Intratracheal		Oral	
	Male	Female	Male	Female
GI Tract (No Bile)	18.2	12.1	73.7	79.0
Bile	19.8	14.5	11.6	9.7
Urine	19.3	13.2	14.6	10.0
Blood[c]	2.2	4.3	1.3	2.8
Spleen	<0.1	<0.1	0.01	0.01
Liver	1.1	1.1	1.0	0.8
Kidney	0.4	0.5	0.2	0.4
Brain	<0.1	0.3	<0.1	0.1
Lung	0.3	0.3	0.4	0.3
Muscle[d]	3.9	8.4	2.0	4.6
Recovered[e]	47.1	45.0		

a. Adapted from El-Hawari et al. 1981.

b. Dose was 50 mg/kg.

c. Based on 7 percent of body weight.

d. Based on 40 percent of body weight.

e. Fat and skin are not included in recovery.

excreting slightly more. Because of the low water solubility of TNT, this radioactivity probably represents metabolic products. Fecal elimination in this study accounted for 2 to 40 percent of radioactivity, but no evidence for elimination via exhalation of CO_2 was found. In another oral exposure study in rats, Ellis et al. (1980) reported only negligible radioactivity (<0.1 percent) in exhaled CO_2. Patterns of elimination similar to those of oral exposures were found for dermal and intratracheal studies (El-Hawari et al. 1981). Because of high amounts of radioactivity in the GI tract of intratracheally exposed rats, bile-duct cannulated rats were studied (Table 19). This study revealed that more radioactivity was released in the bile than in the urine in rats exposed intratracheally as compared to rats exposed orally (El-Hawari et al. 1981). Non-cannulated rats excreted more radioactivity in urine than in bile-duct cannulated rats. After oral dosing, radioactivity recovered in the GI tract represents a balance between absorption, excretion in bile, and intestinal reabsorption. After intratracheal dosing, the radioactivity represents the difference between biliary excretion and intestinal reabsorption with only a small portion of the dose appearing to be excreted through the intestinal wall. This suggests an enterohepatic recycling of TNT and its metabolites (biliary excretion followed by intestinal absorption and final elimination in feces or urine) (El-Hawari et al. 1981).

Studies of the metabolism of TNT have indicated potential pathways and metabolic products, particularly products found in urine. Table 20 lists some metabolic products identified in human and animal studies, and Figure 3 shows some of the potential metabolic pathways that could result in the formation of some of these products. Due to the four functional groups on the TNT molecule, several metabolic reactions are possible (El-Hawari et al. 1981). These include (1) oxidation of the methyl group to alcohol, aldehyde, or acid; (2) reduction of nitro groups to amino or hydroxylamino compounds (with the possible coupling of these metabolites); (3) oxidation of the benzene ring to phenols; and (4) conjugation of these product compounds (acids, amines, alcohols, etc.) to yield glucuronides, ethereal sulfates, substituted hippuric acid, or glutathione conjugates (El-Hawari et al. 1981). The first two reactions are considered the primary metabolic pathways by Ellis et al. (1980). The conjugation to form glucuronides is also an important part of TNT metabolism, and excretion of major portions of TNT metabolites occurs as glucuronide conjugates (El-Hawari et al. 1981). The liver is the primary site for this metabolic and detoxification activity (Zakhari et al. 1978; El-Hawari et al. 1981). Further reductive metabolism of TNT and its metabolites may occur in the intestine by intestinal microflora (Scheline 1973).

4.2 ACUTE TOXICITY

The acute toxicity of TNT has been reported from laboratory tests in animals. The reported oral LD_{50}s of TNT in test animals range from 660 mg/kg in mice to 3190 mg/kg in rats (Table 21), with most values in the 800- to 1000-mg/kg range. Acute effects after dosing in rats and mice centered on nervous system disturbances with inactivity, tremors,

TABLE 19. RECOVERY OF RADIOACTIVITY IN RATS 4 hr AFTER ORAL OR INTRATRACHEAL ADMINISTRATION OF ^{14}C-2,4,6-TRINITROTOLUENE[a]

Tissue or Sample	Percent of Administered Dose[b]			
	Oral		Intratracheal	
	Male	Female	Male	Female
Intact:				
GI Tract	73.70	79.02	18.24	12.06
Urine	14.63	10.01	19.32	13.23
Blood	1.34	2.78	2.24	4.29
Tissue	3.60	6.12	5.80	10.6
Recovered	93.27	97.93	45.60	40.16
Bile-Duct Cannulated:				
GI Tract	68.29	64.22	1.79	2.92
Urine	10.73	8.42	17.50	12.68
Blood	1.34	2.78	2.24	4.29
Tissue	3.60	6.12	5.80	10.58
Bile	11.57	9.67	19.75	14.51
Recovered	95.53	91.21	47.06	44.98

a. Adapted from El-Hawari et al. 1981.

b. Based on mean of four rats at 50 mg/kg.

TABLE 20. METABOLITES OF 2,4,6-TRINITROTOLUENE

Metabolite	Species				
	Human	Rabbit	Rat	Pig Liver	Bacteria
2-Amino-4,6-dinitrotoluene					X[a]
4-Amino-2,6-dinitrotoluene	X[b,c]	X[d]	X[b,e]	X[f]	X[a,g]
6-Amino-2,4-dinitrotoluene	X[b]	X[d]	X[e]		
2,4-Diamino-6-nitrotoluene	X[c]		X[b,e]		X[g]
2,6-Diamino-4-nitrotoluene			X[e]		
4,6-Diamino-2-nitrotoluene		X[h]	X[h]		
Unspecified diaminomononitrotoluene					X[a]
2-Hydroxylamino-4,6-dinitrotoluene		X[i]	X[i]		
4-Hydroxylamino-2,6-dinitrotoluene	X[b]	X[d]			X[a]
2,2',6,6'-Tetranitro-4,4'-azoxytoluene	X[d]				X[a]
2,2',4,4'-Tetranitro-6,6'-azoxytoluene					X[a]
3,5-Diaminonitrobenzene			X[b]		
2,4,6-Trinitrobenzyl alcohol		X[i]	X[i]		
2,4,6-Trinitrobenzoic acid		X[i]	X[i]		
Aminonitrocresols	X[b]		X[b]		
Glucuronides		X[d]	X[e]		
Succinic acid + ammino acids					X[g]
Nitrite, nitrate ions					X[a]

a. Won et al. 1974.

b. Lemberg and Callaghan 1944.

c. Horecker and Snyder 1944, as reviewed in Zakhari et al. 1978.

d. Channon et al. 1944.

e. El-Hawari et al. 1978.

f. Buedding and Jolliffe 1946.

g. Fowler 1965, as reviewed in Dacre and Rosenblatt 1974.

h. El-Hawari et al. 1981.

i. Cited in Rosenblatt 1980; may be formed in one or both species.

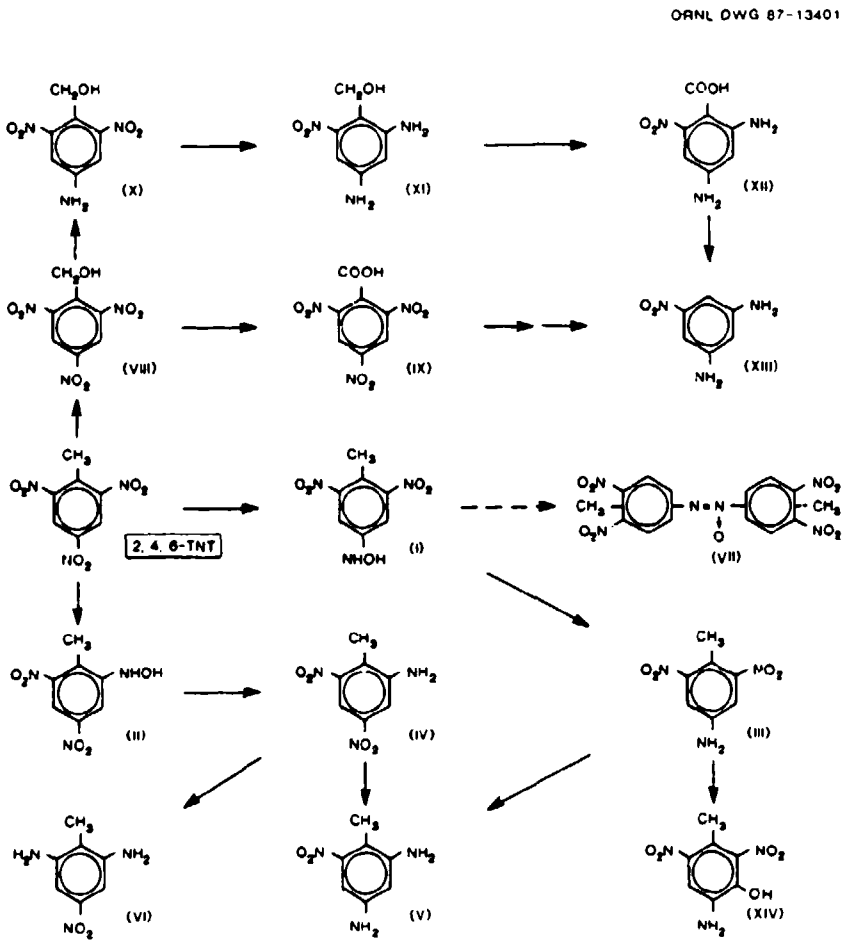

Figure 3. Possible degradation pathway for 2,4,6-2,4,6-Trinitrotoluene.
Source: El-Hawari et al. 1981

(I) 4-Hydroxylamino-2-6-ditnitrotoluene
(II) 2-Hydroxylamino-4,6-dinitrotoluene
(III) 4-Amino-2,6-dinitrotoluene
(IV) 2-Amino-4,6-dinitrotoluene
(V) 4,6-Diamino-2-nitrotoluene
(VI) 2,6-Diamino-4-nitrotoluene
(VII) 2,6,2',6'-Tetranitro-4,4'-azoxytoluene
(VIII) 2,4,6-Trinitrobenzylalcohol
(IX) Trinitrobenzoic acid
(X) 4-Amino-2,6-dinitrobenzylalcohol
(XI) 2,4-Diamino-6-nitrobenzylalcohol
(XII) 2,4-Diamino-6-nitrobenzoic acid
(XIII) 5-Nitro-m-phenylenediamine
(XIV) 4-Amino-2,6-dinitro-m-cresol

TABLE 21. ORAL LD_{50} STUDIES[a] OF 2,4,6-TRINITROTOLUENE

Test Material	Test Species	Sex[b]	LD_{50} Value[c]	Confidence Limits[d]	Reference
TNT[e]	Rat	M	1014±41[f]	922-1108	Lee et al. 1975
TNT	Rat	F	820±32	747-889	Lee et al. 1975
TNT	Rat	NG[g]	3190	NG	Vasilenko and Kovalenko 1976
TNT	Rat	M	1320	955-1824	Dilley et al. 1978
TNT	Rat	F	794	602-1047	Dilley et al. 1978
TNT	Mouse	M	1014±52	905-1163	Lee et al. 1975
TNT	Mouse	F	1009±54	880-1117	Lee et al. 1975
TNT	Mouse	NG	680	NG	Vasilenko and Kovalenko 1976
TNT	Mouse	M+F	830	761-905	Newell et al. 1976
TNT	Mouse	M	660	534-831	Dilley et al. 1978
TNT	Mouse	F	660	574-758	Dilley et al. 1978
TNT	Rabbit	NG	940	NG	Vasilenko and Kovalenko 1976
LAP[h]	Mouse	M+F	1300	110-1500	Newell et al. 1976
SCW[i]	Mouse	M+F	250	180-340	Newell et al. 1976

a. In these studies, oral dosing was by gastric inturbation with test material suspended in corn or peanut oil. Animals were fasted for 16 hours before testing and observed for 14 days post-treatment.

b. M = male and F = female.

c. LD_{50} values are given in mg/kg.

d. Confidence limits are 95 percent.

e. TNT = test material was 99 percent pure α-trinitrotoluene.

f. Plus or minus the standard error.

g. NG = not given.

h. LAP = pink water taken from load, assemble, and pack areas of Joliet Army Ammunition Plant which contained 125.5 ppm of TNT and 30 ppm of RDX.

i. SCW = synthetic condensation water which was generated to simulate actual wastewater by irradiating pure TNT in water thus producing a range of photodegradation products.

and seizures followed by death as the most common effects (Dilley et al. 1978). Respiratory inhibition, cyanosis, and red urine were also noted. If the animals survived several hours after dosing, they usually lived the entire 14-day observation period showing more aggressive behavior but no gross pathological effects. Early studies also indicated anemia and some gross liver damage in guinea pigs, rabbits, and cats (Velling 1943; Bredow and Jung 1942; Haythorn 1920, all as cited in Zakhari et al. 1978).

Studies of the effects of a single 100-mg/kg IP injection of TNT in male rats indicated that enzymatic changes and morphological changes in liver, brain, and kidney tissue occurred (Zitting et al. 1982). Enzymatic effects included increased uridine diphosphate-glucuronosyltransferase activity in liver and kidneys, increased renal epoxide hydrolase activity, decreased succinate dehydrogenase activity in the brain, and increased concentration of reduced glutathione. Morphological damage was more prominent in the brain and kidneys than in the liver. Effects on the brain tissue included chromatin clumping in nuclei of neurons, swelling of cytoplasm in neurons and glial cells, damaged mitochondria, and dilatation of Golgi apparatus. In the liver, the effects were limited to vacuolization of smooth endoplastic reticulum, hypertrophy and elongation of mitochondria, and lipid accumulation in hepatocytes. More prominent effects seen in the kidneys were increased lipid material and lysomes in epithelial cells of proximal tubuli, clumping of nuclear chromatin in epithelial cells, and swollen mitochondria. The effects indicate that peroxidation may be the biochemical cause of the TNT toxicity in this study. A similar study by Tenhunen et al. (1984) indicated additional enzymatic effects on the mitochondrial membranes. Following a 100-mg/kg IP injection in rats, reticulocyte δ-aminolevulinic acid synthetase decreased, liver heme synthetase activity decreased, and liver heme oxygenase activity increased.

4.3 NONCARCINOGENIC CHRONIC TOXICITY

4.3.1 Human Studies

A large literature base, which documents the effects of occupational exposure to TNT on humans, is available. Many of these studies began during and following TNT production for the two World Wars. The primary effects noted in these reports are jaundice with toxic hepatitis and aplastic anemia (Table 22). These effects can occur separately or together and can be fatal. The amount of TNT and the length of exposure required to produce significant effects varied in the studies, and the severity of effects appeared to be more a factor of individual susceptibility. Other effects detected in occupational exposure studies included gastrointestinal disruptions (McConnell and Flinn 1946; Kleiner and Stovpisvskaya 1981), hemolytic anemia (Crawford 1954; Djerassi and Vitany 1975), contact dermatitis (Goh and Rajan 1983; Goh 1984), and cataracts (Hassman 1979).

TABLE 22. SUMMARY OF ANEMIA AND TOXIC HEPATITIS IN HUMANS AS A RESULT OF CHRONIC EXPOSURES OF HUMANS TO 2,4,6-TRINITROTOLUENE

Subject[a]		Exposure[b]	TNT[c]	Primary	
Sex	Number	Age	Duration	Levels	Effects	Reference
M	80	18-70	122 d (avg)	NG[d]	Slight anemia, Hbg <84%	Voegtlin et al. 1921-22
F	39	18-70	122 d (avg)	NG	Slight anemia, Hbg <80%	Voegtlin et al. 1921-22
M	32	18-53	102 d (avg)	NG	Moderate anemia, Hbg = 60 to 71%	Voegtlin et al. 1921-22
F	19	18-53	102 d (avg)	NG	Moderate anemia, Hbg = 60 to 71%	Voegtlin et al. 1921-22
M	1	20	24 d	NG	Severe anemia, Hbg <60%	Voegtlin et al. 1921-22
F,M	5.1	21-42	6 mo	NG	Jaundice, inactive, epigastric pain and anorexia - 1 died with terminal pyrexia	Evans 1941
F	1	35	1 mo	NG	Aplastic anemia, initial jaundice, agranulocytosis, fever - died	Evans 1941
M	1	46	2.5 mo	NG	Jaundice, weakness, shortness of breath, normal blood counts	Palmer et al. 1943
F	1	22	3 mo	NG	Jaundice, weakness, vomiting, shortness of breath, normal blood counts	Palmer et al. 1943
M	1	63	5 mo	NG	Jaundice, epigastric pain, weakness, vomiting, shortness of breath, normal blood counts	Palmer et al. 1943
F	44	20	33 d (avg)	(0.3-0.6[e])	Anemia with decreased hemoglobin, red cells, and hematocrit; increased reticulocytes and serum bilirubin	Stewart et al. 1945
M	8	20	33 d (avg)	(0.3-0.6[e])	Anemia with decreased hemoglobin, red cells, and hematocrit; increased reticulocytes and serum bilirubin	Stewart et al. 1945

TABLE 22. CONTINUED

Subject[a]			Exposure[b]	TNT[c]	Primary	
Sex	Number	Age	Duration	Levels	Effects	Reference
M	10	20	33 d (avg)	(0.3-1.3[e])	Severe anemia with decreased hemoglobin, red cells, and hematocrit; increased reticulocytes, serum bilirubin, and methemoblobin	Stewart et al. 1945
M	1	43	76 d	(1.1-4.2[e])	Initial jaundice, epigastric pain, elevated reticulocyte count - after 3 weeks jaundice was gone, but severe aplastic anemia present leading to death after 75 d - autopsy indicated liver damage (collapsed stoma without hepatic cells)	Sievers et al. 1946
M	1	43	24 mo	(2.1-3.4[e])	Weakness, dizziness, and severe, degenerating, aplastic anemia present leading to death after 43 d - autopsy indicated slight hemosiderosis of liver and bone marrow damage	Sievers et al. 1946
F	1	32	2.5 yr	<0.5[e]	Weakness, dizziness, dysmenorrhea and slight aplastic anemia, recovered following treatment of 5 mo	Sievers et al. 1946
M	6	19-55	39-115 d	NG	Jaundice, high icterus index, occasional anemia leading to death by toxic hepatitis	McConnell and Flinn 1946
F	3	27-30	50-156 d	NG	Jaundice, nausea, high icterus index, occasional anemia leading to death by toxic hepatitis	McConnell and Flinn 1946

TABLE 22. CONTINUED

Subject[a]			Exposure[b]	TNT[c]	Primary	
Sex	Number	Age	Duration	Levels	Effects	Reference
M	11	20-63	73-457 d	NG	Ulcerations and bleeding from several sites, severe aplastic anemia leading to death	McConnell and Flinn 1946
F	2	42-48	98-234 d	NG	Ulcerations and bleeding from several sites, severe aplastic anemia leading to death	McConnell and Flinn 1946
M	1	18	3 d	NG	Weakness, vertigo, nausea, fever with anemia - recovered with steroid treatment; highly susceptible due to glucose-6-phosphate dehydrogenase deficiency	Djerassi and Vitany 1975
M	1	23	4 d	NG	Weakness, vertigo, nausea, fever with anemia - recovered; highly susceptible due to glucose-6-phosphate dehydrogenase deficiency	Djerassi and Vitany 1975
M	1	35	60 d	NG	Weakness, vertigo, nausea, abdominal pain, fever with anemia - recovered; highly susceptible due to glucose-6-phosphate dehydrogenase deficiency	Djerassi and Vitany 1975

a. Data on subjects as given in reports, with F = female, M = male, age given in years

b. Exposure duration as best determined from report, in some cases may only represent length of work history; avg = average.

c. TNT levels as given in report, numbers in parenthesis are ranges.

d. NG = not given.

e. Values are in mg TNT/m^3.

Voegtlin et al. (1921-1922) found a range in the severity of anemic responses (Table 22) to TNT that occurred in over 75 percent of the workers examined. These responses were not related to sex, age, length of exposure, or to occurrence of other clinical signs of TNT intoxication (e.g., cyanosis and pallor). Voegtlin stressed the need to rely on the hemoglobin estimation, because after exposure red blood cell counts are often normal or above normal, due to fragmentation.

Stewart et al. (1945) reported on the early hematological effects of TNT exposure on student workers. They found the same general pattern of effects at all levels with the greatest changes at the highest exposure level (Table 22); individual variation in degree of response occurred. There was little indication of hepatic damage and Stewart et al. speculated that the development of liver damage from TNT required a longer exposure period than did development of hematological effects. The hematological changes (Table 22) were attributed to increased hemolysis and compensation attempts by the bone marrow. With continued exposure, tolerance probably develops in most individuals; if this does not occur, the progressive destruction of circulating erythrocytes and toxic assault on the bone marrow tissue could lead to aplastic anemia (Stewart et al. 1945).

The reports by Sievers et al. (1946) and McConnell and Flinn (1946) both present case histories of workers with aplastic anemia or liver damage (Table 22) usually resulting in death (24 of 25). In the cases described by Sievers et al., hematological changes typical of anemia were first apparent and later developed into aplastic anemia. These were confirmed by bone marrow analysis showing destruction of marrow cells. In only one case was toxic damage to the liver a significant factor. In their summary of case histories, McConnell and Flinn (1946) indicate that a rapid reduction in numbers of red blood cells and in hemoglobin are the primary initial effects noted in the aplastic anemia cases. This diagnosis was later confirmed by hypo- or aplastic appearance of the bone marrow. Based on their small sample size, the authors stated that fatal aplastic anemia rather than fatal hepatitis occurred in older individuals and individuals with longer exposure periods. In addition to the reports summarized in Table 22, several other reports describe hematological effects from exposure to TNT and are reviewed below.

Crawford (1954) reviewed 27 cases of aplastic anemia and 15 fatalities from TNT exposure in the United Kingdom during 1939 to 1945 (Table 23). The effects of TNT exposure on the blood system are variable and may include hemolysis of red cells in the blood stream, destruction of hemoglobin in erythrocytes, formation of methemoglobin, and/or formation of sulfahemoglobin (Crawford 1954). The blood-forming tissues of the marrow are also affected, begining with an increase in numbers of reticulocytes in the blood stream and hyperplasia of bone marrow. With further exposure, the bone marrow becomes hypocellular and the number of red and white cells in the blood are reduced. Progressive aplastic anemia develops with rapid deterioration usually leading to death (Crawford 1954). In the cases reviewed by Crawford, 10 of 15 fatalities also demonstrated liver damage, but no effects were noted in spleen or

TABLE 23. PATTERN OF OCCUPATIONAL EXPOSURE AND DEATHS DUE TO APLASTIC ANEMIA[a]

Duration Variable	Time to Effect (Mo.)					
	Nil	<1	1-3	3-12	12-24	>24
Employment contact with TNT[b]		3 C,[c] 2 D[c]	4 C, 2 D	10 C, 5 D	6 C, 4 D	4 C, 2 D
Last exposure to TNT and start of illness	17 C	6 C	2 C	1 C	1 C	
Duration of illness to date of death		4 C[d]	7 C	4 C	0 C	
to date of resuming work		0 C	3 C	7 C	2 C	

a. Adapted from Crawford (1954) account of fatalities in United Kingdom during during period of 1939-1945.

b. TNT = 2,4,6-Trinitrotoluene.

c. C = number of cases and D = number of deaths.

d. One case died at 9 days and 3 died at 2-4 weeks.

lymph nodes. The ultimate cause of death included 5 cases of intractable recurring hemorrhages, 3 cases of thrombocytopenic purpura, 3 cases of agranulocytosis, 2 cases of brain hemorrhage, 1 case of pulmonary embolism, and 1 case of red cell anemia.

Hathaway (1977) reviewed earlier studies of TNT exposure that resulted in anemic disorders. An Egyptian study by El Ghawabi et al. (1974) examined 38 exposed workers and 20 unexposed controls at a TNT handling plant. The mean exposure levels for most workers were 0.2 to 1.0 mg TNT/m^3. Significant effects ($p < 0.05$) for the exposed group included higher rates of respiratory and gastrointestinal complaints (e.g., coughing or nausea) and a decrease in hemoglobin levels. No changes were seen in liver function tests or in the occurrence of cataracts. A study by the U.S. Army Environmental Hygiene Agency (USAEHA) (Friedlander et al. 1974) also revealed hematologic effects following TNT exposure. Twenty nonexposed workers and 24 workers exposed to TNT levels of 0.03 to 2.52 mg/m^3 were tested for effects. Significantly ($p < 0.05$) lower levels of hemoglobin, hematocrit, and red blood cell counts were found in the exposed group. Also, similarly significant increases in reticulocyte counts, eosinophil counts, blood urea nitrogen levels, and glucose levels were noted. Liver function tests were increased but not significantly. Another, larger USAEHA study examined 533 employees exposed to TNT and 865 not exposed (Buck and Wilson 1975). Although some of the workers were also exposed to RDX, it was felt that exposure should not affect the hepatic and hematological effects from TNT. The 8-hr time weighted average TNT exposures ranged from undetectable (<0.01 mg/m^3) to 1.84 mg/m^3 with only 12.2 percent of the workers exposed to levels >0.5 mg/m^3. Table 24 shows the effects on hemoglobin levels which were the only significant effects even though tests were performed for effects on carbohydrate metabolism, renal function, hepatic function, and the immune system.

Gribova et al. (1983) reported changes in blood parameters that corresponded to increases in TNT exposure concentrations. Examinations of workers exposed for 1 to 20 yr to TNT levels of 0.05 to 7.5 mg/m^3 showed decreased levels of erthyrocyte and hemoglobin concentrations, and increased levels of reticulocytes, erythrocytes, and Heinz bodies. These effects were noticeable at a level of 0.48 mg/m^3 and were more pronounced with higher exposure concentrations. However, there was not a relationship between effects and years of exposure.

At lower TNT exposure levels of 0.10 and 0.35 mg/m^3, nine Finnish workers also exhibited blood-system effects (Savolainen et al. 1985). The activity of aminolevulinic acid synthetase was significantly ($p < 0.01$) lower in exposed workers than in controls. The mean heme synthetase activity was also significantly ($p < 0.05$) lower. None of the tested workers had clinical anemia or morphological damage to microchrondria or bone marrow. This suggests that effects on reticulocytes may occur in the blood after oxygenation.

Morton et al. (1976) studied the effects of low-level TNT exposure (time-weighted average) on serum enzymes and hemoglobin in 43 workers. No significant effects were found for hemoglobin levels, but significant

TABLE 24. RELATIONSHIP BETWEEN HEMOGLOBIN LEVELS AND 2,4,6-TRINITROTOLUENE EXPOSURE[a]

TNT Exposure Level[b]	All Participants		
	No. Abnormal[c]	No. Normal	Relative Odds[d]
None	78	785	1.0
<0.01	24	191	1.3
0.02-0.09	10	63	1.6
0.10-0.19	18	80	2.3
0.20-0.29	4	20	2.0
0.30-0.39	5	5	10.1
0.40-0.49	14	33	4.3
0.50-0.99	3	5	6.1
>1.50	1	1	10.1

a. Adapted from Buck and Wilson 1975, as cited in Hathaway 1977.

b. Time weighted average (8-hr) in mg/m^3.

c. Males: abnormal is less than 14 g Hgb/100 mL blood; females: abnormal is less than 12 g Hgb/100 mL blood.

d. An estimate of relative risk of abnormal hemoglobin with increasing exposure to TNT. In all cases the odds are relative to those not exposed.

changes were found for serum enzyme levels when the TNT levels increased (Table 25). Serum glutamic oxaloacetic transaminase and lactate dehydrogenase activity both increased significantly when exposure increased from 0.3 to 0.8 mg/m^3. These enzymes indicated hepatocellular damage and perhaps some degree of hemolyses (Morton et al. 1976).

Liver damage, the other primary effect from exposure to TNT, has been discussed less frequently in the literature. Evans (1941) summarizes seven cases of liver damage attributed to TNT exposure (Table 22). The initial symptoms included jaundice indicated by yellow color, presence of bile pigments in urine, epigastric pain, and nausea. The one fatal case attributed to toxic liver damage revealed acute yellow atrophy of the liver when autopsied. Palmer et al. (1943) discussed three cases of liver damage from TNT exposure (Table 22). The symptoms were the same as presented in Evans (1941), but recovery occurred in all three cases. The report by McConnell and Flinn (1946) also details eight fatal cases of toxic liver damage from TNT exposure (Table 22). Symptoms observed in most cases included those found in the previous two reports as well as a high icterus index. Deep jaundice, stupor, severe weakness, and other signs of acute toxemia occurred rapidly in these cases with a mean elapsed time of 34 days from initial symptoms to death. Pathological examination revealed reduced liver weight and size, atrophy with complete destruction of parenchymal cells, hemorrhagic areas, perivascular infiltration of lymphocytes and polymorphonuclear leucocytes, and fatty infiltration of cells. Speculation on the mechanism by which TNT produces liver damage was offered by Alperin et al. (1944, as cited in Zakhari et al. 1978); they felt that it was direct action on liver cells. This degeneration is followed by jaundice and destruction of bile capillaries (Cone 1944, as cited in Zakhari et al. 1978).

Another effect documented from chronic occupational exposure to TNT is the formation of cataracts. Hassman (1979) summarizes the early Soviet and East European literature on cataracts associated with TNT worker exposure and also discusses data from an epidemiological survey of Czechoslovakian workers. Reports of formation of cataracts from exposure to TNT first appeared in the literature in 1953 (Glezarov 1953; Holstein et al. 1953, as cited in Hassman 1979). Later reports described the cataracts as a 2-mm-wide ring in the center of the lens usually with an associated peripheral ring (Letavet 1964, as cited in Hassman 1979). The cataract progressed with continued exposure, first appearing after 1 to 2 yr of exposure, but did not affect visual acuity. Occurrence of cataracts in workers varied from less than 1 percent to 81 percent of the exposed population (Penkov 1965, as cited in Hassman 1979). Several authors described the morphological appearance of the cataracts and the stages of progression (Soboleva 1969; Tijukina 1967; Hassman and Juran 1968, 1971, all as cited in Hassman 1979). However, none of these reports detailed the etiology of TNT cataracts nor documented other optical changes in morphology or visual acuity. Hassman also presented data on cataracts found in 54 TNT workers; these data confirmed earlier reports of the morphology and lack of visual deterioration. Hassman indicated that TNT intoxication was not required for the formation of the cataract, because only five individuals of the

TABLE 25. RELATIONSHIP BETWEEN SERUM ENZYME ACTIVITY LEVELS, HEMOGLOBIN LEVELS, AND OCCUPATIONAL 2,4,6-TRINITROTOLUENE EXPOSURE[a]

Parameter[b]	Preemployment	12/73	1/74	2/74	3/74	4/74
Hgb	15.58[c]	14.96	15.19	15.07	14.85	14.90
SGOT	34.78	35.11	32.47	39.06	58.17[d]	54.44[d]
LDH	64.37	52.63	61.72	60.88	105.78[e]	102.76[e]
Avg. TNT Dust (mg/m^3)	NA[f]	0.3	0.3	0.3	0.8	0.6

a. Adapted from Morton et al. 1976.

b. Hgb = hemoglobin;
SGOT = serum glutamic oxaloacetic transaminase;
LDH = lactic dehyrogenase.

c. Values are mean of 43 employees.

d. Significant at $p < 0.01$.

e. Significant at $p < 0.005$.

f. NA = not applicable.

54 examined showed signs of other TNT effects in a battery of blood, enzyme, neurological, and clinical examinations. A similar pattern of cataract formation was also reported for TNT workers in Finland (Harkonen et al. 1983).

4.3.2. Animal Studies

A number of studies have been performed with rats, dogs, mice, and monkeys to examine the effects of TNT following subchronic (13-week) and chronic (26-week to 2-year) exposure. In most cases, similar effects were detected in each study, and these effects paralleled those found in cases of human occupational exposure. These studies are discussed in detail in this section.

Hart (1974) exposed beagle dogs to TNT in the diet for 90 days. TNT levels of 0.02, 0.1, or 1.0 mg/kg/day were fed to three dogs of each sex, and observations were made for clinical, hematological, urinalysis, and biochemical signs of toxic effects. Control animals received similar treatment without exposure to TNT. During the first two-week period, scattered episodes of emesis were noted, but with no discernible pattern. After a 90-day exposure, the dogs were sacrificed, and gross and microscopic pathological examinations of various organs and tissues of high-dosed and control animals were performed. Included in the examinations were hemoglobin estimates, liver enzyme tests, urine bilirubin tests, liver histology, and bone marrow histology. No effects related to TNT treatment were found in the experiments.

Martin (1974) tested the oral toxicity of TNT in cynomolgus monkeys (*Macaca fascicularis*) for 90 days using gastric intubation. Groups of three adult males and three adult females were given doses of 0, 0.02, 0.1, or 1.0 mg/kg/day. Observations for toxic effects included clinical examinations, body weights, hematological tests, clinical chemistry tests, urinalysis, ophthalmoscopic examinations, gross pathology, and histopathology of selected organs and tissues. Effects were limited to the highest dose and included an initial weight loss and presence of hemosiderin in the liver, spleen, and bone marrow. Also, the bone marrow contained necrotic or degenerative megakaryocytes, and two animals had no megakaryocytes in the bone marrow. The authors felt this effect might be related to thrombocytopenia. The lack of dose response in the effects and a failure to detect effects in the hematological and clinical chemistry screens make the significance of the histopathological effects difficult to interpret.

A more extensive evaluation of oral TNT toxicity was reported by Dilley et al. (1978) using 90-day exposures of dogs, mice, and rats. For the dogs, groups of five male and five female beagles were given TNT dose levels of 0, 0.2, 2.0, or 20 mg/kg/day in gelatin capsules. After 4 weeks, one male and one female from each dose group were sacrificed, and one male and one female from each dose group were taken off treatment for 4 weeks (for recovery) and then sacrificed. The remaining animals were kept on test for a total of 13 weeks, and then two males and two females from each dose group were killed. As at 4 weeks, one male and one female from each dose group were set aside for 4 weeks of

recovery and then killed. Observations included complete hematological and biochemical examinations (including interim sampling at 0, 4, 8, 13, and 17 weeks), urinalysis, organ and body weights, gross pathology and histopathology of numerous tissues and organs. The observations included hemoglobin estimates, liver enzyme tests, liver histology, and bone marrow smears. Significant effects for dogs are summarized in Table 26.

The 90-day exposure study of rats and mice used groups of 20 males and 20 females at each dose level with TNT given as a percent of the diet (Dilley et al. 1978). Levels of 0, 0.002, 0.01, 0.05, or 0.25 percent TNT by weight were fed to Sprague-Dawley rats. Based on food consumption and body weight data, these percent levels corresponded to respective doses of 0, 1.40, 6.97, 34.7, or 160 mg/kg/day for males and 0, 1.45, 7.41, 36.5, or 164 mg/kg/day for females. Levels of 0, 0.001, 0.005, 0.025, or 0.125 percent TNT were fed to Swiss-Webster mice. Again based on food consumption and body weight data, these percent levels corresponded to respective doses of 0, 1.45, 7.46, 35.7, or 193 mg/kg/day for males and 0, 1.57, 8.06, 37.8, or 184 mg/kg/day for females. Sacrifice schedules were as follows: five males and five females from each dose level were killed at 4 weeks; another five males and five females from each dose level were removed from TNT exposure at 4 weeks, allowed to recover for 4 weeks and then killed; the remaining rats were kept on test for a total of 13 weeks and then half were killed and half kept for a 4-week recovery before sacrifice. Observations included blood and serum chemistry analysis at each sacrifice time, organ and body weights, gross pathology, and histopathology of numerous tissues and organs. The observations included hemoglobin estimates, liver enzyme tests, liver histology, and bone marrow histology. Significant effects for rats are summarized in Table 27 and for mice in Table 28.

In general, the effects for all three species were similar, with a little more severity noted in dogs and rats (Dilley et al. 1978). Principal effects included depressed weight gain, mild to moderate anemia, enlarged livers and spleens, some testicular atrophy, and hemosiderosis of the spleen. This study parallels many of the human case history reports, indicating anemia (usually hemolytic, but also aplastic) and liver damage as the primary responses.

A further analysis of the subchronic toxicity of TNT to rats was performed by Levine et al. (1981). Groups of 10 male and 10 female Fischer 344 rats were fed diets resulting in TNT doses of 1, 5, 25, 125, or 300 mg/kg/day for 13 wks. A control group of 30 rats of each sex was also included. Observations included general behavior, body weight, food consumption, hematology, clinical chemistry, organ weights, gross pathology, and histopathology. Included in the examinations were hemoglobin estimates, liver enzyme tests, liver histology, and bone marrow histology. The results (Table 29) indicate the principal effects are again on the hepatic and hematological systems. The pattern of hematological effects indicates a more hemolytic rather than an aplastic anemia (Levine et al. 1981). The histopathological effects on the brain and kidneys were not dose-dependent (occurring only at the high dose) and were not supported by any other observations; therefore, their

TABLE 26. SIGNIFICANT TOXIC EFFECTS IN DOGS FROM 90-DAY ORAL EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Parameter	Effects at dose (mg/kg/d)		
	0.2	2.0	20.0
Behavior	NE[b]	NE	Inactivity in males, diarrhea
Body Weight	NE	Initial loss in some	Initial loss in some animals; loss in others late in study - may indicate delayed onset of toxicity
Food Consumption	NE	NE	Lower during first and second week, gradually increasing over treatment
Organ Weights	NE	NE	Enlarged spleen, liver, adrenal and kidney (males only); smaller heart (males only) and testes
Hematology	NE	Mild anemia (decreased hemoglobin, hematocrit, red blood cells), leukocytosis	Pronounced anemia (decreased hemoglobin, hematocrit, red blood cells), leukocytosis
Clinical Chemistry	NE	Decreased iron levels	Decreased SGPT[c] and iron levels; increased cholesterol and bilirubin; effects noted at 4, 8, and 13 weeks, but were reversible
Urinalysis	NE	NE	Dark amber color; high specific gravity
Histopathology	NE	NE	Liver lesions and enlarged livers (at 13 wk); moribund male (at 13 wk) had hyperplasia of bone marrow, extramedullary hematopoiesis, and hyperplasia of prostate; at 17 wk moderate hemosiderosis of the spleen in one female

a. Adapted from Dilley et al. 1978.

b. NE = no effect.

c. SGPT = Serum glutamic pyruvate transaminase.

TABLE 27. SIGNIFICANT TOXIC EFFECTS IN RATS FROM 90-DAY ORAL EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Parameter	Effects at dose (percent w/w)			
	0.002	0.01	0.05	0.25
Body Weight	NE[b]	NE	Small weight loss	Significant[c] weight loss
Food Consumption	NE	NE	Slightly lower than controls during first week and occasionally thereafter	Significantly lower than controls over entire treatment period
Organ Weights	NE	NE	Enlarged liver and spleen in males at 4 wk; decreased testes at 4 wk	Enlarged spleen (at 4 and 13 wk), liver (in males at 4 wk and in females at 13 wk), kidney, and heart; smaller testes (at 4 and 13 wk)
Hematology	NE	Mild anemia in females (decreased hemoglobin, hematocrit, red blood cells)	Mild anemia (significantly decreased hemoglobin, hematocrit, red blood cells)	Pronounced anemia (decreased hemoglobin, hematocrit, red blood cells) at 4 and 13 wk; leukocytosis in males at 4 wk; lymphocytosis at 13 wk
Clinical Chemistry	NE	Decreased iron levels	Decreased iron levels	Decreased SGPT[d] (males), glucose, and iron levels; increased cholesterol (also at 4 wks) and uric acid; effects noted were reversible
Histopathology	NE	NE	Testicular atrophy at 4 wk and 17 wk (4 wk recovery); vacuolated cells in adrenals and nephrosis of kidneys in 3 of 5 males at 13 wk	Testicular atrophy and hyperplasia of interstitial cells at 4 and 13 wks - these were not reversible; hemosiderosis of spleen in both sexes at 4 and 13 wks;

a. Adapted from Dilley et al. 1978.

b. NE = no effect.

c. Significance at $p < 0.05$.

d. SGPT = Serum glutamic pyruvate transaminase

TABLE 28. SIGNIFICANT TOXIC EFFECTS IN MICE FROM 90-DAY ORAL EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Parameter	Effects at dose (percent w/w)			
	0.001	0.005	0.025	0.125
Body Weight	NE[b]	NE	Initial weight loss during first wk, recovered by 2 wk	Initial weight loss during first wk, recovered by 6 wk
Food Consumption	NE	NE	Slightly lower than controls during first week recovered by 2 wk	Slightly lower than controls during first week recovered by 2 wk
Organ Weights	Enlarged spleens at 13 wk; enlarged hearts in males at 13 wk	Enlarged hearts in males at 4 wk	NE	Enlarged spleen (in males at 4 wk) at 13 wk; enlarged liver (in males at 17 wk) enlarged heart at 13 wk in males;
Hematology	NE	NE	NE	Mild anemia (decreased hemoglobin, hematocrit, red blood cells) at 4 wk; only slight decrease in hematocrit and red blood cell in males at 13 wk
Histopathology	NE	NE	NE	Hemosiderosis of spleen in both sexes at 13 wks

a. Adapted from Dilley et al. 1978.

b. NE = no effect.

TABLE 29. SIGNIFICANT TOXIC EFFECTS TO RATS FROM 13-WEEK DIET EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Parameter	Effects at dose (mg/kg/day)				
	1	5	25	125	300
Body Weight Gain	NE[b]	10% reduction in males	10% reduction in males	10% reduction in females; 24% reduction in males; ($p < 0.05$)[c]	38% reduction in females; 46% reduction in males; ($p < 0.05$)
Food Consumption	NE[d]	NE	NE	Slight reductions in females, possible reduction in males	Slight reductions in females, possible reduction in males
Hematology	NE	NE	Anemia with decreases in hemoglobin, hematocrit, red blood cells (significant in males, $p < 0.05$)	Anemia with significant decreases in hemoglobin, hematocrit, red blood cells ($p < 0.05$)	Anemia with significant decreases in hemoglobin, hematocrit, red blood cells ($p < 0.05$); also statistically significant formation of methemoglobin ($p < 0.05$)
Clinical Chemistry	NE	NE	Slight elevations of serum cholesterol	Significant elevations of serum cholesterol ($p < 0.05$)	Significant elevations of serum cholesterol ($p < 0.05$)
Organ Weights	NE	Enlarged liver and spleen but not significant	Enlarged liver and spleen but not significant	Enlarged liver and spleen ($p < 0.05$)	Enlarged liver and spleen ($p < 0.05$); testicular atrophy ($p < 0.05$)
Histopathology	NE	NE	Mild, diffuse sinusoidal congestion in spleen in 1 male; degeneration of epithelium lining in testes of 1 male	Splenomegaly with mild and diffuse sinusoidal congestion in spleen of 1 male and 4 females; degeneration of epithelium lining in testes of 6 males; multifocal or diffuse hepatocytomegaly seen in 6 males; brown pigment in renal tubular epithelium cell cytoplasm of most animals	Splenomegaly with moderate to severe sinusoidal congestion in spleen of 9 males and 10 females; degeneration of epithelium lining in testes of 10 males; brain lesion seen in 6 males and 3 females; multifocal or diffuse hepatocytomegaly seen in 10 males and 8 females; brown pigment in renal tubular epithelium cell cytoplasm of all animals

a. Adapted from Levine et al. 1981.

b. NE = minor effect, 5% reduction in males.

c. Statistically significant

d. NE = no effect.

significance must be questioned when extrapolating to human exposure to TNT. Testicular damage similar to that in the Dilley et al. (1978) study occurred; however, its significance for humans is uncertain, because testicular atrophy is a common response in rats to any stress or toxic insult and may not reflect a TNT-specific effect (Dilley et al. 1978). Levine et al. (1981) suggest that the testicular damage is secondary to an altered androgen metabolism.

Levine et al. (1983) followed up the 13-wk rat study with a 26-wk study of TNT in the beagle dog. Groups of six dogs of each sex were given capsules containing 0, 0.5, 2.0, 8.0, or 32.0 mg/kg/day. Observations included general behavior and clinical examination, body weight changes, food consumption, complete hematological, clinical chemistry, and urinalysis screening, ophthalmological examinations, gross pathology, and histological examination of more than 30 organs and tissues. Blood samples were taken every 4 to 5 weeks for the laboratory tests, but interim sacrifices were not made. The examinations included hemoglobin estimates, liver enzyme tests, liver histology, and bone marrow smears. Results are shown in Table 30. Toxic effects from oral exposure to TNT in the beagle are very similar to those found in the rat. The major effects from TNT exposure in this study are dose-dependent hemolytic anemia and dose-dependent hepatic damage. The anemia was accompanied by a physiological compensation response including reticulocytosis and macrocytosis. However, unlike the study in rats (Levine et al. 1981), no histologic evidence of damage to the testes, brain, or kidneys was found in dogs.

A 2-yr study in Fischer 344 rats was performed by Furedi et al. (1984) to evaluate the chronic toxicity and carcinogenicity of TNT in the diet. Rats were fed diets containing 0, 0.4, 2, 10 or 50 mg/kg/day for 24 months, and a range of observations was made including pathological observations of interim sacrifices at 12 months. Effects at the 10- and 50-mg/kg/day levels included weight loss, decreased food consumption, anemia with compensatory reticulocytosis, splenic lesions (hemosiderosis and sinusoidal congestion), liver damage (hepatomegaly with hepatocellular hyperplasia), and altered lipid and protein metabolism. Also, at the highest dose, renal damage occurred with increased blood urea nitrogen (BUN) levels, increased kidney weight, and hyperplasia of the renal pelvis. At the 2.0-mg/kg/day level, indications of splenic lesions, renal pigmentation (in females), and some bone marrow damage occurred, although statistically significant anemic responses were not found. The 0.4-mg/kg/day level failed to produce toxic effects and was considered a no-observed-effect level.

4.4 GENOTOXICITY

A variety of short-term test systems has been used to evaluate the genotoxicity of TNT and TNT-complexes with most systems indicating positive effects. The Ames *Salmonella typhimurium* assay is a well-established genotoxic test system and was used by several researchers to test TNT, the urine of workers in TNT production facilities, and TNT-surfactant complexes for genotoxic potential.

TABLE 30. SIGNIFICANT TOXIC EFFECTS IN DOGS FROM A 26-WEEK ORAL EXPOSURE TO 2,4,6-TRINITROTOLUENE[a]

Parameter	Effects at dose (mg/kg/day)			
	0.5	2.0	8.0	32.0
General Observations	NE[b]	NE	NE	Ataxia, jaundice; 2 deaths preceded by diarrhea and an icteric state
Body Weight Gain	Reduction, but not significant[c]	Reduction, but not significant	Significant reduction in males	Significant reduction
Food Consumption	NE	NE	Slight reduction in females during 1st wk	Significant reduction
Hematology	Anemia with nonsignificant decreases in hemoglobin, hematocrit, red blood cells	Anemia with nonsignificant decreases in hemoglobin, hematocrit, red blood cells	Anemia with significant decreases in hemoglobin, hematocrit, red blood cells; also significant formation of methemoglobin	Anemia with significant decreases in hemoglobin, hematocrit, red blood cells; also significant formation of methemoglobin
Clinical Chemistry	NE	Decreased SGPT[d] (significant in females)	Significant decreased SGPT	Significant decreased SGPT and increased LDH[e]
Urinalysis	NE	Possible increased bilirubin levels	Significant increases in bilirubin levels	Significant increases in bilirubin levels
Ophthamology	NE	NE	NE	NE
Organ Weights	NE	Enlarged liver and spleen but not significant	Enlarged liver (significant only in males) and spleen (significant only in females)	Significantly enlarged liver, spleen, and kidneys (only in females)
Histopathology	Hepatocytic cloudy swelling, 7 of 12 dogs showed trace to mild lesions in the liver (hepatocytomeglia)	Hepatocytic cloudy swelling, mild lesions in the liver (hepatocytomeglia), and hemosiderosis in liver cells in 1 female; enlarged spleen and splenic hemosiderosis	Hepatocytic cloudy swelling, mild to moderate lesions in the liver (hepatocytomeglia), and hemosiderosis in liver cells of all dogs; enlarged spleen and splenic hemosiderosis; reduced myeloid:erythroid ratio	Hepatocytic cloudy swelling, moderate to severe lesions in the liver (hepatocytomeglia), hepatic cirrhosis in all males, and hemosiderosis in liver cells of all dogs; enlarged spleen and splenic hemosiderosis; reduced myeloid:erythroid ratio

a. Adapted from Levine et al. 1983.

b. NE = no effect.

c. Significant or nonsignificant refers to significance levels of $p < 0.05$.

d. SGPT = serum glutamic pyruvate transaminase.

e. LDH = lactate dehydrogenase.

Won et al. (1976) exposed the *S. typhimurium* strain TA98 to various concentrations of TNT in an overlay plate test, with and without S9 activating enzymes. At concentrations greater than 10 g/mL, growth inhibition occurred. At 0.5 to 10 g/mL, a dose-dependent increase in the frameshift mutation rate occurred in a linear fashion. The genotoxic effects only occurred in tests without S9 activation, suggesting that enzymatic action on TNT produces only nonmutagenic metabolites.

Dilley et al. (1978) tested TNT dissolved in dimethyl sulfoxide on strains TA98, TA100, TA1535, TA1537, and TA1538 of *S. typhimurium* at doses of 10, 50, 100, 500, 1000, and 5000 mg TNT/plate. In tests without S9 activation, a TNT dose of 100 mg/plate produced a twofold to sixfold increase in the mutation rate of strains TA98, TA100, and TA1538. At 500 mg/plate, significant increases were also found in strain TA1537, but higher doses produced toxicity. In S9-activated tests, doses lower than 100 mg/plate did not produce any increase in mutation rates, and, at 500 mg/plate, only the mutation rate in strain TA100 was significantly affected. However, at 1000 mg/plate, significant increases were again seen in the other four strains.

Whong and Edwards (1984) repeated Ames tests of TNT using the five basic strains at doses of 110, 220, 440, 660, and 880 nanomoles/plate, without S9 activation. An increase in mutation frequency was not seen in strain TA1535 at any dose. However, in the other strains, TA98, TA100, TA1537, and TA1538, a clear dose-response pattern was established. Tests with S9 activation failed to increase mutation frequencies.

A study by Sundvall et al. (1985) of the genotoxic effects of munition wastewaters indicated that TNT was genotoxic to *S. typhimurium* strain TA100. Of the 10 various genotoxic components of the wastewater, TNT was the second highest contributor to the overall genotoxic response, being responsible for 10 percent of the total.

In addition to reports of the genotoxicity of pure TNT, several Swedish researchers examined the urine of TNT production workers for effects. Ahlborg et al. (1985) reported a study of Swedish TNT workers in which an alternative to the conventional Ames plate incorporation test with *S. typhimurium* strain TA98 was used. A bacterial fluctuation assay developed by Green et al. (1976, as cited in Ahlborg et al. 1985) indicated increased frameshift mutations when tested without S9 activation. Urine samples from 14 workers produced significant increases ($p < 0.01$), and these increases could be correlated with observed TNT levels in the ambient air of the work stations.

The genotoxic potential of TNT coupled with surfactants used to remove TNT from wastewater streams was evaluated by Kaplan and Kaplan (1982) using the Ames assay. The surfactants were Duoquad T-50 (N-tallow-N'N'N'-trimethyl-N,N-dimethyl-1,3-diamino-propane), Duomeen T (N-tallow-1,3-diamino-propane, white waxy solid), and Arquad T-50 (N-tallow-trimethylammonium). The three complexes were toxic to *S. typhimurium* at doses of 5000 g/plate, and all produced higher mutation rates than TNT alone at equivalent concentrations. TNT was mutagenic to strains TA1538, TA100, and TA98 at concentrations of 50 to 500 g/plate,

while the surfactants were mutagenic in all strains at concentrations 100-fold lower.

Other assays of the genotoxic potential of TNT were reported. In a short abstract, Geshev and Kincheva (1974) report that 6 months of topical application of 30 percent TNT to rats resulted in chromatid changes and chromosome breaks in the bone marrow in half of the test animals.

Dilley et al. (1978) reported two genotoxic studies of TNT. The first study was an *in vivo* cytogenic analysis of bone marrow, and the second was an *in vitro* analysis of unscheduled DNA synthesis (UDS) in human diploid fibroblasts. Doses used in the rat study were 0.25 and 0.002 percent TNT in the diet and the rats were fed for 28 days. Upon sacrifice and examination, no cytogenic abnormalities or chromosome losses were found in the bone marrow samples. Because microbial studies showed that mutations should occur, the authors suggested that the lack of effects *in vivo* may indicate insufficient amounts of TNT were ingested or the TNT was deactivated in the metabolic pathways of the rats. In the human UDS study, cell cultures of human fibroblasts were inoculated with dilutions of a TNT solution and compared to control values. At a concentration of 1000 g/mL without metabolic activation, significant elevations occurred in the incorporation rate, indicating genotoxic effects. However, color interference at the two higher doses prevented the determination of a dose-response pattern, and thus UDS was only suggested in this assay.

Styles and Cross (1983) reported the results of a P388 mouse lymphoma gene mutation assay of TNT. Cells in growth cultures were exposed to TNT concentrations of to 1000 g/mL, with and without S9 activation. TNT in two trials caused a significant increase in mutation frequency and dose-related cytotoxicity, but only in unactivated systems. This test also underscores that metabolic activity deactivates the genotoxicity potential of TNT; Styles and Cross speculated that *in vivo*, TNT would be hepatotoxic but not genotoxic.

Ashby et al. (1985) evaluated the genotoxicity of TNT in two assays, a mouse bone marrow micronucleus test and an *in vitro/in vivo* rat liver assay for UDS. Male hybrid mice were injected IP with 10 mL/kg of a TNT solution at doses up to 80 percent of the maximum tolerated dose and observed for 4 days. Slides of bone marrow were examined from five animals at intervals of 24, 48, and 72 hr after dosing. At doses of 40 and 80 mg/kg, neither a significant increase in bone marrow micronuclei nor a dose response pattern was observed in this assay. In the rat UDS assay, male Alderley Park or Fischer 344 rats were given TNT by gavage, and then 12 hr later hepatocytes were isolated for UDS analysis. Results for TNT doses of up to 1000 mg/kg were negative in both strains of rats.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

Information on the reproductive and developmental effects of TNT was very limited. Zakhari et al. (1978) in a review of the literature

reported that no teratogenic studies had been performed. Our searches also failed to locate any reports whose endpoint was solely developmental toxicity or reproductive effects. As discussed earlier, in subchronic and chronic toxicity studies by Levine et al. (1981; 1984) testicular atrophy with degeneration of seminiferous tubular epithelium was seen in rats after 13-wk exposures to TNT levels of 125 and 300 mg/kg/day. However, as noted by Dilley et al. (1978) and Levine et al. (1981), the significance of these effects is questionable, because of a failure to demonstrate similar effects in other species and because this pattern of effects is a common response of rats to any toxic insult.

4.6 CARCINOGENICITY

As with developmental/reproductive toxicity, limited data were available on the carcinogenic effects of TNT. Zakhari et al. (1978) in their literature review reported that no occupational evidence of carcinogenicity was available and no post-mortem tumors had been found. In reports of short-term genotoxic assays, some comments were made on the possible carcinogenicity of TNT. Styles and Cross (1983), in their discussion of positive genotoxic effects of TNT in the mouse lymphoma cell assay, suggest that some concern is warranted regarding the potential carcinogencity of TNT in the liver. However, they cautioned that the biotransformation of TNT is different *in vitro* from *in vivo* and that the potential for hepatocarcinogenicity may not be supported in whole animal feeding studies. Ashby et al. (1985), in discussing the negative responses of TNT in mouse bone marrow micronucleus and rat liver UDS tests, also discounted the possible liver carcinogenicity of TNT. Perhaps a weak carcinogenicity response would be elicited from the systemic effects on the hemopoetic or nephro-urinary systems at toxic doses (Ashby et al 1985); but at lower levels this possibility is remote.

A 2-yr study of a TNT-diet exposure of rats has been reported (Furedi et al. 1984; Levine pers. comm. 1986). In the 2-yr study, Fischer 344 rats were fed diets containing 0, 0.4, 2.0, 10, or 50 mg/kg/day and observed for chronic toxic effects and for potential carcinogenicity. Effects at the 10- and 50-mg/kg/day levels included weight loss, decreased food consumption, anemia with compensatory reticulocytosis, splenic lesions (hemosiderosis and sinusoidal congestion), liver damage (hepatomegaly with hepatocellular hyperplasia), and altered lipid and protein metabolism. Also at the highest dose, renal damage occurred with increased BUN levels, increased kidney weight, and hyperplasia of the renal pelvis. Hyperplasia (12 of 47 animals) and carcinoma (11 of 47 animals) of the urinary bladder were observed at significant levels ($p < 0.01$ and $p < 0.05$, respectively) in high-dose females. A dose-response trend was not observed for the carcinogenic effects, although one or two females at 10 mg/kg/day were affected. In their discussion, the authors indicated that the hyperplasias in the liver, kidneys, and urinary bladder support the concept of carcinogenicity of TNT to the Fischer 344 rat under the conditions of this study (Furedi et al. 1984).

4.7 SUMMARY

Trinitrotoluene is absorbed by both humans and test animals through the skin, by ingestion, and by inhalation. The amount absorbed varies among different routes and test species; absorption was slower by the dermal route than by the intratracheal and oral routes. Following oral absorption, ^{14}C-TNT is widely distributed throughout the body at low levels. The highest levels are found in the GI tract, liver, kidneys, and blood. After 24 hr, the majority of radioactivity from ^{14}C-TNT exposure by any route was found in the urine and feces of all species. Although experiments were not taken beyond 24 hrs, there is an indication that retention of ^{14}C-TNT is not extensive. Because of the low solubility of TNT in water, radioactivity in urine probably represents metabolic byproducts. No evidence for elimination via exhalation of CO_2 was found.

Studies of the metabolism of TNT have indicated potential pathways and byproducts, particularly products found in urine. Due to the four functional groups on the TNT molecule, several metabolic reactions are possible. These include the primary metabolic pathways of (1) oxidation of the methyl group to alcohol, aldehyde or acid; and (2) reduction of nitro groups to amino or hydroxylamino compounds (with the possible coupling of these metabolites). The liver is the site for this metabolic and detoxification activity.

The reported oral LD_{50}s of TNT in test animals range from 660 mg/kg in mice to 3140 mg/kg in rats, with most values in the 800- to 1000-mg/kg range. Acute effects after dosing in rats and mice centered on nervous system disturbances with inactivity, tremors, and seizures followed by death. Respiratory inhibition, cyanosis, and red urine were also noted.

A large literature base, documenting the effects of occupational exposure to TNT, is available. The primary effects noted in these reports are jaundice with toxic hepatitis and aplastic anemia. These effects can occur separately or together and can be fatal. The amount of TNT and the length of exposure required to produce significant effects varied in the studies, and the severity of effects appeared to be a factor of individual susceptibility. Other effects detected in occupational exposure studies include gastrointestinal disruptions, hemolytic anemia, contact dermatitis, and cataracts. The effects of TNT exposure on the blood system are variable and may include hemolysis of red cells in blood stream, destruction of hemoglobin in some erythrocytes, formation of methemoglobin, and formation of sulfhemoglobin. The blood-forming tissues of the marrow are also affected, beginning with an increase in numbers of reticulocytes in the blood stream and hyperplasia of bone marrow. With further exposure, the bone marrow becomes hypocellular, and the number of red and white cells in the blood are reduced. Progressive aplastic anemia develops with rapid deterioration usually leading to death. Occupational studies that provided exposure levels indicated that significant effects on the hematological system

(primarily decreased hemoglobin) occurred at mean exposure levels of 0.2 to 7.5 mg/m^3.

Monkeys, that were given doses of 0, 0.02, 0.1, or 1.0 mg/kg/day for 90 days using gastric intubation, showed effects that were limited to the highest dose and that included an initial weight loss and presence of hemosiderin in the liver, spleen, and bone marrow. Also, the bone marrow had necrotic or degenerative megakaryocytes, and two animals had no megakaryocytes in the bone marrow. A more extensive evaluation of oral TNT toxicity was reported using 90-day exposures of dogs, mice, and rats. The dogs were given TNT dose levels of 0, 0.2, 2.0, or 20 mg/kg/day in gelatin capsules. Levels of 0, 0.002, 0.01, 0.05, or 0.25 percent TNT by weight were fed in the diet to rats. Levels of 0, 0.001, 0.005, 0.025, or 0.125 percent TNT were fed to mice. In general, the effects for all three species were similar, although slightly more severe in dogs and rats. Principal effects included depressed weight gain, mild to moderate anemia, enlarged livers and spleens, some testicular atrophy, and hemosiderosis of the spleen. This study parallels many of the human case history reports, indicating anemia (usually hemolytic, but also aplastic) and liver damage as the primary responses. A similar pattern of effects were found in a 13-wk rat diet study (TNT doses of 1, 5, 25, 125, or 300 mg/kg/day) and in a 26-wk beagle dog study (given capsules with 0, 0.5, 2.0, 8.0, or 32.0 mg/kg/day). The pattern of anemic effects indicated a hemolytic basis rather than an aplastic anemia.

A variety of short-term test systems has been used to evaluate the genotoxicity of TNT and TNT-complexes, with most systems indicating positive effects. The Ames *Salmonella typhimurium* assay was used to test TNT and the urine of workers in TNT production facilities. Tests of pure TNT in *S. typhimurium* strains TA98, TA100, TA1537, and TA1538 demonstrated increased frequencies of mutations, usually in the absence of S9 activating enzymes. Levels of 100 to 1000 mg/plate, 0.5 to 10 g/mL, and 110 to 880 nanomoles/plate produced effects in most strains, frequently in dose-dependent patterns. A study of Swedish TNT workers presented an evaluation of genotoxicity using a bacterial fluctuation assay which indicates increased frameshift mutations when tested without S9 activation. Urine samples from 14 workers revealed significant increases ($p < 0.01$) in mutations, correlated with observed TNT levels in the ambient air of the work stations. Other evaluations of genotoxicity of TNT included a cytogenic analysis of bone marrow, unscheduled DNA synthesis (UDS) assays in rat liver cells and human diploid fibroblasts, a P388 mouse lymphoma gene mutation assay, and a mouse bone marrow micronucleus assay. Only in the human UDS assay (at 1000 g/mL) and the mouse lymphoma assay (at doses from 0 to 1000 g/mL) were significant genotoxic effects reported.

No data were found on the teratogenic or developmental toxicity of TNT in mammals. The only indication of reproductive effects were isolated effects on testes of rodents during chronic tests. Carcinogenicity data were limited to a 2-yr study of rats which indicated hyperplasia and carcinoma of the urinary bladder in females at the highest dose (50 mg/kg/day), but without a dose-response pattern.

5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

Standards for TNT exposures in work situations have been set by the Occupational Safety and Health Administration (OSHA), and recommendations have been made by the U.S. Army. A threshold limit value (TLV) of 1.5 mg/m^3 was established for TNT by OSHA (Morton et al. 1976). However, Morton et al. also reported serum enzyme changes at levels below this TLV and indicated that the U.S. Army Environmental Hygiene Agency was considering a level of 0.5 mg/m^3 for TNT exposures at Army facilities. The recommendation for an 8-hr time weighted average of 0.5 mg/m^3 was also made by Hathaway (1977). Zakhari et al. (1978) reported that maximum allowable concentrations of TNT in the USSR. and Czechoslovakia are 1.0 mg/m^3. They also reported that the U.S. Army had officially lowered its standard to 0.5 mg/m^3 in November 1976 (DARCOM Regulation 40-3, November 1976, as cited in Zakhari et al. 1978). The TLV of 0.5 mg/m^3 was endorsed by the ACGIH (1983), and they also suggested a short-term exposure limit (STEL) of 0.3 mg/m^3. Limits for TNT in drinking water were reported for the Navy Bureau of Medicine (0.05 mg/L) and the U.S. Army (0.03 mg/L) (Committee on Toxicology 1982). A U.S. Army limit for TNT in wastewater (0.01 mg/L) was also reported (Committee on Toxicology 1982).

5.2 OCCUPATIONAL EXPOSURE

Many reports of occupational exposure to TNT have been made in the literature, including reports of fatalities. Primarily these exposures occurred during and following the first and second World Wars, when production was high and knowledge of the health effects was incomplete. These reports are discussed in Sections 4.3.1 and 4.4 and indicate that the primary effects of inhalation of TNT are hepatic damage and anemia at reported atmospheric concentrations of 0.10 mg/m^3 and higher.

5.3 PREVIOUSLY CALCULATED CRITERIA

Previous attempts have been made to calculate water quality criteria to protect human health and aquatic life using the then current guidelines of the U.S. Environmental Protection Agency.

Dilley et al. (1978) in their report of acute and subchronic toxicity of TNT to mammals calculated acceptable daily intakes (ADIs) for humans and a maximum recommended concentration of TNT in water. Based upon their studies of dogs, rats, and mice, Dilley et al. generated ADIs of 0.2, 1.42, and 7.76 µg/kg/day, respectively. These values were generated using a no-observed-effect level (NOEL) for each species and dividing by an uncertainty factor of 1000. These ADIs were then inserted into the following equation to generate maximum concentrations:

$C = ADI \times 70/(2 + 0.0187R)$, where R = bioconcentration factor,

70 = avg weight in kg for human, and 2 = average intake of water in L.

To obtain a value for R, Dilley et al. used the estimated oil/water partition coefficient for TNT and determined a value of 11.5, which when substituted in the above equation gives maximum concentrations of 6.3, 44.7, and 245 μg/L for TNT, based on dog, rat, or mouse data, respectively. Thus, in this estimate there was nearly a 40-fold range in ADI depending on the species used.

Rosenblatt (1981) calculated the maximum allowable concentrations and the estimated water quality criterion for TNT released from Savannah Army Depot Activity. He used U.S. Environmental Protection Agency (1980) guidelines and estimates for total TNT concentration in fish, total fish consumption, and bioconcentration factor of TNT. The estimates based on noncarcinogenic data gave an acceptable daily dose for TNT of 1.4 μg/kg and a drinking water criterion of 50 μg/L. Using a safety factor of 100 to account for the potential carcinogenicity of TNT, Rosenblatt estimated a daily acceptable dose of 0.014 μg/kg and a drinking water criterion of 0.5 μg/L. After later review of the data and procedures used to calculate the criteria, the values were considered as rather uncertain (Rosenblatt 1986, pers. comm.).

Dacre (1980) in a letter to the Office of the Surgeon General reported interim environmental criteria based on rat and bioconcentration data from Dilley et al. (1978) and using the 1979 USEPA guidelines. With a bioconcentration factor of 11.5 and a NOEL of 1.4 mg/kg/day (based on 90-day rat studies) used to calculate an acceptable daily intake, an interim criterion of 44.25 μg/L was recommended for the protection of human health. Data were not sufficient to allow the U.S. Army to calculate criteria to protect aquatic organisms, but a value of 60 μg/L was suggested, based on preliminary results from fathead minnow studies. For regulating drinking water, a similar level of 44.0 μg/L was suggested in a letter to the Office of the Surgeon General (U.S. Army 1983).

Bailey et al. (1985), in a report on the chronic toxicity of TNT to several species of fish, also calculated water quality criteria based on USEPA (1980) guidelines. They used data they generated from early life history and chronic studies, as well as data from Liu et al. (1983). With these data, Bailey et al. determined a Final Acute Value (FAV), Final Chronic Value (FCV), and Final Plant Value (FPF), although these determinations were done without meeting all of the requirements or assumptions stated in the USEPA (1980) guidelines. They also calculated a Final Residue Value (FRV) based on an estimate of the bioconcentration factor using the log P estimate for TNT and the USEPA (1980) equation. Their calculated values were 0.9 mg/L for the Final Acute, 0.05 mg/L for the Final Chronic, 4.1 mg/L for the Final Plant, and 24.4 mg/L for the Final Residue. Because their data for chronic effects indicated that effects occurred at a level of <0.04 mg/L, they replaced the calculated Final Chronic Value with this lower estimate. Thus, using the USEPA (1980) guidelines, Bailey et al. recommended a maximum allowable concentration of 40 μg/L and a 24-hr average concentration of 40 μg/L as their aquatic criteria. They also cautioned that the 24-hr average might produce some effects, as it was not a NOEL.

5.4 AQUATIC CRITERIA (See Appendix A)

The aquatic criteria consist of two values, a criterion maximum concentration and a criterion continuous concentration (Stephan et al. 1985). The criterion maximum concentration is calculated by dividing the Final Acute Value by two. The criterion continuous concentration is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value. Data available for calculating aquatic criteria for TNT do not meet all the requirements specified by the latest USEPA guidelines (Stephan et al. 1985), thus strictly speaking one should not calculate aquatic criteria. However, an attempt was made to generate interim water quality criteria using the formula provided in the USEPA guidelines.

The data for the Final Acute Value are insufficient; available tests do not include enough genera (only 7 of the required 8 genera) and not all tests were performed under flow-through conditions. However, data on an additional, unrequired genus were available, and the guidelines do make provisions for the use of static tests if performed under acceptable standards; therefore, a Final Acute Value was calculated (Table 31). The criterion maximum concentration based on this FAV is 557 μg/L.

Data for calculating a Final Chronic Value are also insufficient. Enough information exists in the report by Bailey et al. (1985) to calculate 1 of the 3 required acute-to-chronic ratios (for *Salmo gairdneri*) using early life-stage toxicity studies. A 3-generation chronic study of *Pimephales promelas* performed by Bailey et al. did not determine a level at which adverse effects did not occur, consequently, the data in this study cannot be used to determine an acute-to-chronic ratio. However, based on the lowest chronic effect level for reproductive effects on *P. promelas*, a tentative estimation of the criterion continuous concentration might be 40 μg/L. This estimation is lower than the lowest effect level of 240 μg/L observed to significantly reduce fry survival in *S. gairdneri* and should be an acceptably conservative estimate until a NOEL is generated for *P. promelas* by further study.

Data for a Final Plant Value are available for a vascular plant exposed for 11 days and an algal species exposed for 21 days. Schott and Worthley (1974) found that a level of 1.0 ppm reduced growth in a duckweed species. Bailey (1982) found that levels of 0.6 to 1.0 mg/L produced reductions in cell densities in the alga *Selenastrum capricornutum* in a pattern that indicated the threshold effect might be in that range. Since none of these values are lower than the 40 μg/L level found in the chronic animal studies, one could assume that aquatic plants would be protected by any level determined, based on chronic aquatic animal data.

A Final Residue Value cannot be calculated because data on maximum permissible tissue concentrations are not available. Data for bioconcentration factors (BCF) were also not acceptable. Data from a study by Liu et al. (1983) are not acceptable because the exposure was only 4 days (versus 28 days required) and the measured values were therefore,

TABLE 31. CALCULATIONS FOR FINAL ACUTE VALUE (FAV) OF 2,4,TRINITROTOLUENE[a]

Rank (R)	GMAV[b]	ln GMAV[c]	$(\ln GMAV)^2$	P=R/(N+1)[d]	$\sqrt{P}$
4	2.81	1.0332	1.0675	0.4444	0.6666
3	2.78	1.0224	1.0453	0.3333	0.5773
2	2.72	1.0006	1.0012	0.2222	0.4714
1	1.34	0.2927	0.0857	0.1111	0.3333
Sum:		3.3489	3.1997	1.1111	2.0486

a. Based on calculation methods discussed in Stephan et al. (1985).

b. GMAV = genus mean acute value in mg/L; calculated as the geometric mean of LC_{50} values (N = 8) meeting necessary standards; listed in Table 4.

c. ln GMAV = natural log of GMAV.

d. P = probability for each GMAV; R = rank of four lowest GMAVs; N = 8.

$$S^2 = \frac{\Sigma[(\ln GMAV)^2] - (\Sigma(\ln GMAV)^2/4)}{\Sigma(P) - (\Sigma\sqrt{P})^2/4}$$

$$L = [\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P}))]/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

$$S^2 = \frac{3.1997 - (3.3489)^2/4}{1.1111 - (2.0486)^2/4} = \frac{0.3959}{0.0619} = 6.3958$$

$$S = 2.5290$$

$$L = [3.3489 - (2.5290)(2.0486)]/4 = -0.4580$$

$$A = (2.5290)(\sqrt{0.05}) - 0.4580 = 0.1075$$

$$FAV = e^{0.1075} = 1.1135 \text{ mg/L}$$

probably not at steady-state. Data from a study by Xu and Chen (1983) are not acceptable because the species studied are not native North American species. However, calculations based on octanol-water partition coefficient data indicate that TNT is not likely to bioconcentrate (Cairns and Dickson 1978). Therefore, the lack of acceptable data on BCFs is not crucial, and any calculation of the criterion continuous concentration would most likely be based on the determination of a Final Chronic Value.

5.5 HUMAN HEALTH CRITERIA (See Appendix B)

Data on TNT have not shown any significant carcinogenic pattern; data suggest the possibility of carcinogenicity, but a dose-response pattern was not established. Also, occupational exposure histories have not indicated a correlation between TNT exposure and any significant carcinogenicity. Reliable evidence from human exposures to quantify definite concentrations of TNT were not available for calculating a human health criterion. The most extensive study reporting effects for TNT was the 2-yr diet study using rats (Furedi et al. 1984). They found some pathological effects at a dose level of 2.0 mg/kg/day and established a NOEL of 0.4 mg/kg/day. Based on USEPA (1980) guidelines, an uncertainity factor of 100 is required for chronic animal data, and the 0.4-mg/kg/day value is therefore adjusted by a factor of 100. The resulting value of 0.004 mg/kg/day is multiplied by 70 kg to give a human ADI (acceptable daily intake) of 0.28 mg/day. Insertion of this ADI into the human health criterion equation gives the following:

$$C = \frac{[ADI - (DT + IN)]}{[2L + 0.0065\ kg \times BCF)]},$$

$$C = \frac{[0.28 - (0 + 0)]}{[2L + (0.0065\ kg \times 11.5)]},$$

where,

C = criterion,
DT = non-fish dietary intake,
IN = inhalation intake,
2L = daily water intake in liters,
0.0065 kg = daily dietary fish intake, and
BCF = bioconcentration factor,

gives a human health criterion of 134.96 $\mu g/L$. The non-fish dietary and inhalation intakes were assumed to be zero, based on physical/chemical properties and occupational hygiene practices. The bioconcentration factor was assumed to be 11.5 based on the calculations with the octanol-water partition coefficient of Dilley et al. (1978).

5.6 RESEARCH RECOMMENDATIONS

The following research recommendations are based on the 1980 and 1985 USEPA guidelines and would be required to properly calculate water quality criteria. Recommended tests should meet all protocol standards as outlined in Stephan et al. (1985) and USEPA (1980).

1. An additional flow-through, acute study with measured concentrations is needed on a species of aquatic insect or in a phylum not already represented (see Table 4) (e.g., an Ephemeroptera or Mollusca) in order to calculate an acute-to-chronic ratio for aquatic organisms.

2. Steady-state or 28-day bioconcentration studies are needed using flow-through tests with measured concentrations of TNT.

3. Chronic studies of TNT using a life-cycle toxicity test in *Daphnia magna* and *Pimephales promelas* under flow-through conditions and measured TNT concentrations. A definitive no-observed-effect level should be generated for reproductive effects and effects on the second generation.

4. A chronic study of TNT effects on beagle dogs with enough dose levels to determine dose-response might be considered, because of the greater sensitivity demonstrated in 26-wk studies (definite effect level of 0.5 mg/kg/day) as compared to chronic studies in rats (slight effect level of 2.0 mg/kg/day and no-effect level of 0.4 mg/kg/day). Although not strictly required by USEPA guidelines, additional knowledge and safety provided by such a study might justify such an effort.

5. Examination of the potential for carcinogenic effects suggested by the urinary bladder carcinomas in female rats at the high dose as reported in Furedi et al. (1984). This is the only indication of carcinogenicity of TNT, but further study could determine if human health criteria should be based on non-threshold effects. Similarly, a chronic dog and/or rat study could be designed to also address this topic.

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7. GLOSSARY

AAP	Army Ammunition Plant
ADI	Acceptable daily intake
BCF	Bioconcentration factor (R)
BOD	Biological oxygen demand
BUN	Blood urea nitrogen
COD	Chemical oxygen demand
DMSO	Dimethyl sulfoxide, a common vehicle for test chemicals
dpm	Disintegrations per minute
EC_{50}	Effective concentration based on immobilization
FAV	Final acute value
FCV	Final chronic value
FEL	Frank effect level
FPV	Final plant value
FRV	Final residue value
GC	Gas chromatography
GI	Gastrointestinal
GMAV	Genus mean acute value
gpm	Gallons per minute
Hgb	Hemoglobin
IP	intraperitoneal injection
LAP	Load, assemble, and pack
LC_{50}	Lethal concentration to 50 percent of test organisms
LD_{50}	Lethal dose to 50 percent of test organisms
LDH	Lactate dehydrogenase
NOAEL	No observed adverse effect level
NOEL	No observed effect level

NPDES	National Pollution Discharge Elimination System
OSHA	Occupational Safety and Health Agency
ppb	Parts per billion
ppm	Parts per million
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine; cyclotrimethylene trinitrotriamine
SGOT	Serum glutamic oxaloacetic transaminase
STEL	Short-term exposure limit
TLV	Threshold limit value
TNT	α-Trinitrotoluene (unless mentioned otherwise)
UDS	Unscheduled DNA synthesis
USAEHA	U.S. Army Environmental Hygiene Agency
USAMBRDL	U.S. Army Medical Bioengineering Research and Development Laboratory
USATHAMA	U.S. Army Toxic and Hazardous Materials Agency
USEPA	U.S. Environmental Protection Agency
UV	Ultraviolet radiation

APPENDIX A

SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect aquatic life with emphasis on the specific regulatory needs of the U.S. Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C. E. Stephan, D. I. Mount, D. J. Hansen, J. H. Gentile, G. A. Chapman, and W. A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species at all times was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species is very sensitive. The small fraction is set at 0.05, because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from

acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors, such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived; however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative; much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

3. Definition of the material should include an operational analytical component. It is also necessary to reference or describe analytical methods that the term is intended to denote. Primary

requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants; (b) FDA action levels (FDA Guidelines Manual); and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.

2. All data used should be available in typed, dated, and signed hard-copy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.

3. Questionable data, whether published or not, should not be used.

4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.

5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentration of test material in test solutions were measured using acceptable analytical methods.

6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:

a. the family Salmonidae in the class Osteichthyes;

b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish);

c. a third family in the phylum Chordata (e.g, fish or amphibian);

d. a planktonic crustacean (e.g, cladoceran or copepod);

e. a benthic crustacean (e.g, ostracod, isopod, or amphipod);

f. an insect (e.g., mayfly, midge, stonefly);

g. a family in a phylum other than Arthropoda or Chordata (e.g, Annelida or Mollusca); and

h. a family in any order of insect or any phylum not represented.

2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.

3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.

4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly, if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if sufficient acute and chronic data are available, it may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).

3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L), should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.

5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:

   a. Tests with daphnids and other cladocerans should be started with organisms <24 hr old, and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} is not available from a test, the 48-hr LC_{50} should be used in place of the desired 48-hr EC_{50}. An EC_{50} or LC_{50} of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

   b. The result of tests with all other aquatic animal species should be the 96-hr EC_{50} value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} value is not available from a test, the 96-hr LC_{50} should be used in place of the desired EC_{50}.

   c. Tests with single-cell organisms are not considered acute tests, even if the duration was $\leq$96 hr.

   d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.

6 If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV, because a species can only be considered protected from acute toxicity if all life stages are protected.

8. Consider the agreement of the data within and between species. Questionable results in comparison with other acute and chronic data for the species and other species in the same genus probably should not be used.

9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentrations of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

   NOTE: Data reported by original investigators should not be rounded off, and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low, and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

12. Calculate the cumulative probability (P) for each GMAV as R/(N+1).

13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).

14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^{A}$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.

16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.

4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

5. Similarly normalize the values of the water quality characteristic for each species individually.

6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).

9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

10. For each species calculate the SMAV using: $SMAV = e^{Y}$.

11. Obtain the FAV at Z by using the procedure described in Section 5 (Nos. 10-14).

12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.

13. The Final Acute Equation is written as: $FAV = e^{(V[\ln(\text{water quality characteristic}) + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

   NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.

3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.

5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

   a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young <48 hr old, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young <24 hr old and last for not <21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.

   b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

   c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

      NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be

used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used, because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.

7. If chronic toxicity of the material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.

8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species, and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (Nos. 10-14). Then, go to Section 7 (No. 13).

9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used or acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.

10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or

decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.

b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and saltwater species.

c. If the most appropriate species mean acute-chronic ratios are <2.0, and especially if they are <1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and an FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.

13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.

14. Go to Section 9.

8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.

a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z (see Section 6, No. 13) by the Final Acute-Chronic Ratio.

c. Use V = pooled acute slope (see Section 6, No. 13) as L = pooled chronic slope.

d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.

a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.

c. Decide whether data for each species are useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.

d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

e. Similarly normalize the values of the water quality characteristic for each species individually.

f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality

characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.

i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^{Q}$).

k. Obtain the FCV at Z by using the procedure described in Section 5 (Nos. 10-14).

l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.

m. The Final Chronic Equation is written as: $FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$, where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.

2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.

3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which

concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11, because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.

3. If a maximum permissible tissue concentration is available for a substance (e.g, parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

   a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if

apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.

b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.

c. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.

d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, in which case the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

   a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.

   b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.

   a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage

of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.

b. Calculate the geometric mean normalized BCF.

c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.

- For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.
- For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).
- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.

3. The Criterion Continuous Concentration (CCC) is equal to the lowest of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final

Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.

4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as:

   The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) µg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) µg/L more than once every three years on the average.

   Here,

   (1) = insert freshwater or saltwater,

   (2) = insert name of material,

   (3) = insert the Criterion Continuous Concentration, and

   (4) = insert the Criterion Maximum Concentration.

13. REFERENCES

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APPENDIX B

SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final US Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect human health and is slanted towards the specific regulatory needs of the U.S. Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

a) Carcinogenicity,
b) Toxicity, and
c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the values represent estimates that would prevent adverse health effects, or for suspect and proven carcinogens, estimates of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAH HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes, including ingestion of contaminated water and edible aquatic and nonaquatic organisms as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish.

Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to be insignificant; however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions, which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).

2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.

3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used, depending upon lipid solubility and availability of bioconcentration data:

1. For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980).

   Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}} ,$$

   where BCF_{sp} is the bioconcentration factor for an aquatic species and PL_{sp} is the percent lipids in the edible portions of that species.

2. Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

   for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent

lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

3. For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects, is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to 10^{-5} (one additional case of cancer in populations ranging from ten

million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific lifetime carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065\ BCF)}$$

where

- C = ambient water concentration;
- PR = the probable risk (e.g., 10^{-5}; equivalent to one case in 100,000);
- BCF = the bioconcentration factor; and
- q_1^* = a coefficient, the cancer potency index (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = \frac{q_1^* \times C\ (2 + 0.0065\ BCF)}{70}$$

or

$$PR = \frac{q_1^* \times 2C + (0.0065\ BCF \times C)}{70}$$

where 2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

Simplified terms the equation can be rewritten

$$PR = q_1^* X ,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X} ,$$

showing that the coefficient q_1^* is the ratio of risk to dose, an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5}, which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared with the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then RR(X) = 5). In such cases the "excess" relative cancer risk is expressed as RR(X) - 1, and the actual numeric, or proportional, excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(control) .$$

Using the standard risk/dose equation

$$PR(X) = b \times X$$

and substituting for PR(X):

$$[RR(X) - 1] \times PR(control) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(control)}{X} ,$$

where b is equal to the carcinogenic potency or q_1^*.

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.

2. The data set giving the highest index of carcinogenic potency (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.

4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the 2/3 power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W}$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3} .$$

3. The dose must also be normalized to a lifetime average exposure. For a carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is l_e, and the total length of the experiment is L_e, then the lifetime average exposure (X_m) is

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}} .$$

4. If the duration of the experiment (L_e) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of $(L/L_e)^3$ to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = ppm \times F \times r ,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man; 0.05 for a 0.35-kg rat; and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3} .$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol and is absorbed proportionally to to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption, which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp\{-g(d)H(t)\},$$

where P(t;d) is the probable response for dose d and time t, g(d) is the polynomial function defining the effect of dose level, and H(t) the effect of time:

$$g(d) = \sum_{i=0}^{a} \alpha_i d^i$$

$$H(t) = \sum_{i=0}^{b} \beta_i t^i$$

(with α and $\beta \geq 0$, and $\Sigma \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each α as a multiplicative constant (Crump 1980):

$$p(d/t) = 1 - \exp\left(- \sum_{i=0}^{a} \alpha d^i \right) ,$$

or as given in the EPA guidelines (USEPA 1980):

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where P(d) is the lifetime risk (probability) of cancer at dose d.

For a given dose the excess cancer risk A(d) above the background rate P(o) is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

where,

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \ldots + q_k d^k)] .$$

Point estimates of the coefficients $q_1 \ldots q_k$ and consequently the extra risk function A(d) at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function A(d) has approximately the form:

$$A(d) = q_1 \times d .$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R. Thus, A(d) and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1. This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^*, the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_0 - L_1) = 2.70554 ,$$

where L_0 is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$\chi^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1 - P_i)}$$

where Ni is the number of animals in the ith dose group, Xi is the number of animals in the ith dose group with a tumor response, Pi is the probability of a response in the ith dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (χ^2) is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at and below which the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065\ kg \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criterion.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

(1) NOEL = No-Observed-Effect-Level,
(2) LOEL = Lowest-Observed-Effect-Level,
(3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
(4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.

2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.

3. If an LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to an NOAEL or an LOAEL.

4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only an NOEL and an LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, an NOAEL, an LOAEL, and a clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.

2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals, with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported, but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criterion (using the uncertainty factor approach). Also, the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to

those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = \frac{TLV \times BR \times DE \times d \times A_A}{(A_O \times SF)}$$

where

BR = daily air intake (assume 10 m^3),
DE = duration of exposure in hours per day,
d = 5 days/7 days,
A_A = efficiency of absorption from air,
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = \frac{CA \times D_E \times d \times A_A \times BR \times 70\ kg}{BW_A \times A_O \times SF)}$$

where

C_A = concentration in air (mg/m^3),
D_E = duration of exposure (hr/day),
d = number of days exposed/number of days observed,
A_A = efficiency of absorption from air,
BR = volume of air breathed (m^3/day),
70 kg = standard human body weight,
BW_A = body weight of experimental animals (kg),
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may, under some circumstances, result in depressed fluid intake which, in

turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant; however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

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